Antibody-Antigen recognition

Structural Biology Weekend Seminar

10.07.2005

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Basics of antibodies 1

- Antibodies = Immunoglobulins;
- 5 classes: IgA, IgD, IgE, IgG, IgM
- Homodimers
- 2 identical polypeptide chains (450 aa) \(\Rightarrow\) heavy chains
- 2 identical polypeptide chains (250 aa) \(\Rightarrow\) light chains
- Heavy & light chains divided into N-terminal variable & C-terminal constant parts
Basics of antibodies 2

- **β-sheets** together form a barrel-shaped structure ▶️
  - β-barrel

- structure of the immunoglobulin protein domain ▶️
  - immunoglobulin fold
Basics of antibodies 3

- Each heavy chain: $V_H$, $C_H1$, $C_H2$, $C_H3$ domains
  - each domain: 2 anti-parallel $\beta$-sheets

- Each light chain: $V_L$, $C_L$ domains

- Each $V_H$ and $V_L$: 3 loops connecting the $\beta$-strands, highly variable in length & sequence
  - complementarity determining regions (CDRs)
Basics of antibodies 4

- **Fragments antigen binding** (**Fab**): 2 identical fragments complete light chains + VH + CH1
- **Fragment crystallizable** (**Fc**): paired CH2 + CH3
- **Fragment variable** (**Fv**): heterodimer VH + VL
Basics of antibodies 5

- Disulfide bridges link the hinge regions of the heavy chains & $C_{H1} + C_{L}$
Central paradigm of antigen-antibody recognition

The 6 CDRs come together to form a binding surface

3 dimensional structure formed by the 6 CDRs recognizes & binds a complementary surface (epitope) on the antigen

Sequences & structures of the CDRs determine the specificity of the surface
Features of antibody-antigen interfaces 1

• Richer in aromatic residues, esp. tyrosine, tryptophan than average protein surface

• Depleted in charged residues aspartate, glutamate, lysine, but enriched in arginine & aromatic residues

• Analysis of alanine mutations on binding energies → thermodynamic hot spots due to multiple favorable interactions
Features of antibody-antigen interfaces 2

- Light and heavy chains make contacts with antigen (heavy chain more extensive)

- **Specificity** of binding determined by CDRs of $V_H$ and $V_L$

- Contacting residues of the antigen: discontinuous in sequence, but form an epitope

- High **complementarity** of contacting surfaces
Features of antibody-antigen interfaces 3

- Surface areas of interaction: ca. $600-900\text{Å}^2$

- **Mediation** of binding: v.d.Waals, hydrogen bonds, salt bridges, occasional ion pairs

- **Water molecules** reinforce complementarity & interactions (supported by molecular dynamics simulations)

- **Enthalpic forces** (hydrogen bonds, v.d.Waals) drive reaction
Binding of a small antigen

- Complex between an Fab fragment of an antibody & its target phosphoryl-choline
- Residues from the antibody interact through H-bonding & electrostatic & v.d. Waals interactions
Antibody-Protein Interactions

- Complex between an Fab fragment + **lysozyme**
- Binding surfaces are complementary in shape over a large area
- Glutamine 121 penetrates more deeply into the antibody combining site
Fv--Mouse anti-HEL monoclonal Antibody D1.3 complex with lysozyme

- 1.8 Å resolution
- 178 water molecules, 50 around interface with HEL
- Small conformational changes /side chain movements esp. in $V_H$ CDR3
- Small rearrangement of $V_H$ and $V_L$ domains → contacting residues closer to antigen by 0.5-0.7 Å

→ “induced fit“ facilitating binding of antigen
Mouse anti-HEL (hen egg-white lysozyme) monoclonal Antibody D1.3

Stereo view of the VL CDR1 and CDR2 (thick bonds) & neighboring solvent molecules & lysozyme atoms from the Fv D1.3-HEL complex

► extent of hydration at the interface
Mutant of Fv D1.3 ($V_L$ Trp92→Asp) complexed with HEL

- 1000-fold reduction in equilibrium binding constant

- \textbf{wt}: Trp92 $\triangleright$ extensive v.d.Waals with HEL Gln121, Arg125 & Ile124

- \textbf{Mutant}: rearrangement of H$_2$O molecules at $V_L$-HEL interface $\triangleright$ 2 H$_2$O molecules fill hole created by Trp92→Asp

- Contact area lost: 150 Å$^2$ $\triangleright$ -16 kJ mol$^{-1}$ enthalpy
Mutant of Fv D1.3 ($V_L$ Trp92→Asp) complexed with HEL.
Mouse anti-HEL Fab D44.1

- 3 salt links between $V_H$ glutamates (35 & 50) and HEL arginines (45 & 68)

- Arg45 steric hindered by $V_L$ Trp94 from its preferred rotameric position

- 3 $H_2O$ molecules buried at interface ➔ H-bonds, stability & complementarity of complex
Stereo view H-bonding scheme between Fab D44.1 and HEL

Fab D44.1 (thick bonds) and HEL (lighter bonds);

stacking of guanidino group of HEL Arg68 & indole ring of V_{H} Trp54

Antibody-Antigen Recognition
Cross-reactivity

• Cross-reactions with closely related antigens frequently observed

• Antibodies binding better to antigens not used in challenging immune system
  ➤ heteroclitic binding

• Anti-HEL monoclonal antibody D11.15 which was raised against HEL, binds several avian lysozymes with higher affinity
Fv of Anti-HEL D11.15 complexed with PHL (pheasant egg-white lysozyme)

- Comparison with D1.3-HEL
  - Structural model to explain high specificity (D1.3) & broader cross reactivity (D11.15)

- D1.3 and D11.15
  - Obtained from the same individual mouse
  - Bind to partially overlapping epitopes
Fv of Anti-HEL D11.15 complexed with PHL compared with D1.3-HEL

- Replacement at position 121, located in aromatic pocket between $\text{V}_H$ & $\text{V}_L$ domains
  ✓ prevents D1.3 from binding other lysozymes

- Tight fit in a central location of interface
  ➔ D1.3 cannot bind lysozymes not featuring Gln121

- D11.15 binds an epitope shared by several lysozymes

- Here high specificity & cross reactivity depend on location of evolutionarily produced sequence changes in antigen
Modeling of antibody-protein antigen interactions

- Statistical method for assessing the degree of complementarity (defined by packing density)

- Computer-aided modeling of antibody-antigen docking
  - steric score (complementarity & docking)
  - modified Lennard-Jones potential, consisting of a broad energy minimum simulating the likelihood of side-chain reorientation during binding

- Results marginal revealed by crystalline complexes
Other attempts at computer simulation of antibody-antigen docking 1

- Based on maximizing the surface area of interaction

- Docking arrangements showed only limited success
Other attempts at computer simulation of antibody-antigen docking

• Molecular dynamics analysis of D1.3-HEL association

• Many H₂O molecules were attracted to antibody-antigen interface during the case of simulation

• Resulting models do not differ significantly from those obtained from X-ray crystal structures
Anti-idiotypic antibodies (Ab2)

• ...recognize an idiotope (=antigenic determinant that is unique to an antibody (Ab1))

• Aim: Use of these mimics as sort of vaccines
Complex between FabD1.3 & its idiotypic antibody FabE225

- First crystallographic analysis of idiotypic-anti-idiotope interactions

- 14 CDR residues from FabE225 interact with 10 CDR residues from FabD1.3

- 1 framework residue from FabE225, 3 framework residues from FabD1.3
References


Thank you for your attention