

# Chapter 5 Protein function

## Problems 1, 3, 4, 5, 6,

### 5.0 Introduction

As said previously there are thousands of proteins structures now known. In labs will look at a few. Biggest conceptual problem is one you see a 3D structure and use computer to move it around you tend to think protein is a solid brick. Is really more like jello, it moves and wiggles and jiggles. And these moves and wiggles are critical to how it works

In this chapter focuses on details of two are three protein to illustrate the wide range of things that can be done to make proteins work. I will just do hemoglobin and myoglobin

Some key words/concepts

Often proteins will reversibly bind some other molecules or proteins  
we call these molecules **ligands**

The site where it binds is called the **Binding site**

Usually complementary to ligand in some way including size,  
shape, charge, hydrophobic or  
depending on how carefully designed may be extremely selective or  
somewhat promiscuous in selectivity

Protein are flexible, may see subtle changes where just one or two AA rotate slightly, or may see massive movements of entire chunks of protein. Will often talk of protein as 'breathing'

Binding of a ligand often accompanied by change in protein structure that may make binding even better (like folding around) Structural adaptation called **induced fit**

Structural change accompanying binding of 1 ligand often effect binding of other ligand, and this is used to regulate activity of protein as a whole

Enzymes (next chapter) are a special case of protein function. Not only do the bind ligands but they make then go through chemical reactions. In Enzyme we will call the ligands by a special name, **substrates** and we give the binding site a special name, **catalytic site**. Otherwise is the same thing, binding, specificity, conformational change So are learning the basic rule of how protein work, in next chapter will add chemistry into the mix

### 5.1 Reversible binding of protein to ligand O<sub>2</sub> binding proteins

Myoglobin and hemoglobin are perhaps best studied and best understood proteins so will look at them

### A. O<sub>2</sub> bound by heme prosthetic group

O<sub>2</sub> not very soluble (.035g/L) need higher conc  
also need to diffuse over distances > a few mm

No AA's are good O<sub>2</sub> binders

But metals like Fe and Cu are good O<sub>2</sub> binders

But free Fe and O<sub>2</sub> and H<sub>2</sub>O makes hydroxyl radicals that are bad  
for cell

Need to bind Fe away from H<sub>2</sub>O

Iron frequently bound by heme prosthetic group

**Prosthetic group** compound permanently associated with a  
protein that contributes to protein's function

Heme **Figure 5-1**

Organic structure called protoporphyrin ring

Flat, planar, looks aromatic, binds a single Fe<sup>2+</sup>

Fe want 6 coordinate bonds, find 4 in heme ring

Coordination helps prevent oxidation to Fe<sup>3+</sup>

Found in many oxygen bind protein as well as many redox  
proteins

Free heme will bind oxygen, but then Fe oxidizes to +3 state

In protein keep ½ of heme covered, also sequester O<sub>2</sub>  
pocket makes it much harder to oxidize Fe

Binding of O<sub>2</sub> changes electronic properties of heme (as well  
as protein structure)

These account for color change in blood

Other molecule also bind to heme, CO and NO. CO binds  
better than O<sub>2</sub> that is why is poison But protein  
structure helps to favor O<sub>2</sub> over CO binding so not  
quite as overpowering! (More details later)

### B. Myoglobin (Mb) (figure 5-3)

16,700 153 AA's, single heme

found all mammals, primarily in muscle tissue store O<sub>2</sub> for use when  
needed

Particularly in deep sea mammals like whales

member of globin protein family

contains 8 helices that account for about 80% of structure

### C. Quantitative description of Protein ligand interaction

need to look at binding behavior first before we look at atomic explanation  
of behavior

Will use math to measure binding and describe how it works

Binding process is equilibrium process so let's look at equilibria



$K_{\text{ass}}$  or  $K_{\text{binding}}$  won't call  $K_a = [PL]/[P][L]$

Have seen this kind of K before. Now something sort of new

Often useful to think about fraction of protein molecules that have ligand bound

Call this  $\theta$   $\theta = [PL]/[\text{total}] = [PL]/([P]+[PL])$

From above equation  $[PL] = K_a[P][L]$ , substitute

$$\theta = K_a[P][L]/([P]+K_a[P][L])$$

Dividing through by P

$$\theta = K_a[L]/(1+K_a[L])$$

Dividing through by  $K_a$

$$\theta = [L]/(1/K_a + [L])$$

$$\text{Or } [L]/([L]+1/K_a)$$

Might recognize this as a hyperbolic function

$$Y = X/(X+k)$$

Looks like **figure 5-4**

Note special point, when  $\theta = .5 = [L]/2[L]$ , so  $[L] = 1/K_a$

Can also define as  $K_{\text{diss}} = K_D$

$$[PL] \rightleftharpoons [P] + [L]$$

$$K_D = [P][L]/[PL] = 1/K_{\text{ass}}$$

(You should remember this from Gen chem)

So a simpler looking  $\theta$  is:

$$\theta = [L]/(K_{\text{dis}} + [L]) \text{ and when } \theta = .5, [L] = K_{\text{diss}}$$

Note at  $[L] < K_{\text{dis}}$  protein rapidly give up ligand

but at  $[L]$  about  $5X K_{\text{dis}}$  protein is saturated and cant bind any more

#### D. Protein structure affect ligand binding

now have math to describe binding, now look at protein  
Complicated system ,will see lots of different stuff going on, some very subtle

for instance said earlier CO bind to heme better than O<sub>2</sub>

Lets look at quantitatively

For free heme  $K_{dis}$  CO is 20,000X smaller for CO than for O<sub>2</sub>

Meaning it binds CO 20,000 x better!

In protein  $K_{dis}$  is only 200X smaller for CO than O<sub>2</sub> so cut down affinity for CO by 100!

How? Binding pocket not straight. This reinforces the binding of O<sub>2</sub> that prefers to bind at an angle, but interferes with binding of CO that want to bind straight (figure 5-5)

How does it get in an out to begin with. If look at X-ray no hole big enough!!

Protein breathing, as flexes open up hole and gaps on 10<sup>-9</sup> time scale to let in and out

#### E. Hemoglobin O<sub>2</sub> transport in blood

Nearly all O<sub>2</sub> in animals carried by hemoglobin in blood, specifically in red blood cells (erythrocytes)

##### Do I want this??

Erythrocytes

6-9µm biconcave disks

Derive from hemoblast stem cell

Have large amounts of hemoglobin (34% of mass)

Have lost nucleus, mitochondria, and ER

Only last about 120 days

##### Detail on next page, this is an Intro

Mb with hyperbolic binding curve good for holding O<sub>2</sub>

Hb, is a multimer and has a sigmoidal binding curve better suited for transport where has to bind and give off (more in a bit)

In lungs have 96% saturation so up here on curve

in return at 64% saturation so loses about 1/3 of O<sub>2</sub> that it carries

100 mL of blood carries about 6.5 ml of O<sub>2</sub> gas

#### F. Hemoglobin units are similar to Mb units

Hemoglobin (HB) 64,500 MW, 4 hemes, tetrahedral arrangement of 4 Mb like monomers (figure 5-10)

2 alpha chains (141 residues)

2 beta chains (146 residues)

Only about 1/2 of residues are same between MB and Hb, yet structure almost identical (figure 5-6)

Only 27 residue are identical in all three

See figure 5-7

In Hb tetramer are many interaction between units

$\alpha_1\beta_1$  30 residues need urea to break apart

$\alpha_1\beta_2$  19 residues

Lots of hydrophobic interaction, but some Ion pair and H bonds at interfaces

### G. Structural change in Hb on binding O<sub>2</sub>

Observe 2 structures for hemoglobin, depending on if binds O<sub>2</sub> or doesn't

Call state observe without O<sub>2</sub> the T state or 'tense' state

Call state observe when O<sub>2</sub> bind the R state or 'relaxed' state

Tense & relaxed are old, irrelevant term refer to fact that T state has more intersubunit salt bridges so is more tensely (tightly) held together

In R state conformation is shifted so Hb binds O<sub>2</sub> better (higher affinity) than T state

Binding of O<sub>2</sub> to T state triggers conformational change to R state

Transition involves minor change protein structure, fairly large changes in interface contacts

Figure 5-11

Binding of O<sub>2</sub> flattens heme, moves His, moves helix

### H. Hemoglobin Binds Cooperatively

Net effect (Draw on board -then show figure 5-12)

Since T state low binding has this curve with  $\theta=.5$  at high pO<sub>2</sub>

R state is high affinity so  $\theta=.5$  at low pO<sub>2</sub>

As molecules move from one to other get sigmoidal kid of curve

This is called **cooperativity**, binding on one molecules affect binding on another

In lungs, high O<sub>2</sub> high affinity, sucks up all the O<sub>2</sub> it can

In tissue, low O<sub>2</sub> lower affinity, doesn't hold as tightly, gives off O<sub>2</sub> to tissue

Hemoglobin is an example of an **allosteric** protein

Binding of one ligand to one site affects binding at another site

Allostery can be positive or negative

Hemoglobin considered **homotropic** because modulator = ligand  
 Many cases of **heterotropic** modulator  $\neq$  ligand  
 Sometimes more than 1 modulator

Cooperative binding frequently observed in multimeric proteins  
 Allosteric, multimeric proteins are seen often in regulatory proteins

### I. Describing with math

Now let's extend earlier math for n ligand binding sites



$$K_a = [PL_n]/[P][L]^n$$

With a little math

$$\theta = [L]^n / ([L]^n + K_d)$$

Rearranging

$$\theta / (1 - \theta) = [L]^n / K_d$$

And

$$\text{Log}(\theta / (1 - \theta)) = n \text{log}([L]) - \text{log} K_d$$

Why do this? Its called a Hill Plot

See figure 5-14

Shows cooperativity, conc where cooperative, how many cooperative units?

(Don't sweat the math, I don't anticipate any Hill plot problems, other than looking at a plot and interpreting)

### J. Models for cooperative binding

2 main models have been proposed to explain cooperativity

MWC (Monod, Wyman + Changeux 1965)

Sequential (Koshland 1966)

Figure 5-15

MWC. All molecule in T or R

Sequential, 1 molecule can have both T and R

See all the equilibria?

Make equation to describe all equilibrium

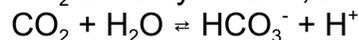
Bottom line, data yet to be good enough to say which is correct!

### K. Hb also transports H<sup>+</sup> & CO<sub>2</sub>

Now let's get more complicated

What else is in the blood? CO<sub>2</sub>

CO<sub>2</sub> not very soluble, body makes soluble by converting to carbonic acid



A spontaneous but slow reaction, body want fast so can reverse reaction can go forward it tie blood is in tissue and completely reverse in time blood is in lung, have special enzyme Carbonic Anhydrase one of the fastest enzymes known

“But wait there's more”

Net effect, in peripheral tissue H<sup>+</sup> high, pH lower

Body uses this effect to help unload even more O<sub>2</sub>

Called the Bohr Effect **Figure 5-16**

Higher [H], lower pH, less O<sub>2</sub> bound

Effect primarily comes from changing ionization state of several ionizable groups on Hb molecule Which one do we expect (his) you are right His 146

Hb also carries CO<sub>2</sub> as carbamino group on terminal NH<sub>2</sub>

See **right column page 166**

Net Hb not only carries O<sub>2</sub> but about 20% of CO<sub>2</sub> and H<sup>+</sup> generated in peripheral tissue

### L. O<sub>2</sub> binding further regulated by 2,3 biphosphoglycerate (BPG)

**Structure left hand column 167**

BPG 5mM sea level, 8mM high altitude

Sea level control is for about 40% of max to be delivered to lungs however move to 4500 m (15,000 ft) only have about ½ as much O<sub>2</sub> in air, so cannot deliver as much

BPG conc increases, makes HB have less affinity so kick off more O<sub>2</sub>

So continues to deliver about 40%

**See figure 5-17**

Also another story in hemoglobin in fetal tissues, need and α<sub>2</sub>γ<sub>2</sub> Hb in fetus, because must take O<sub>2</sub> from mother H<sub>b</sub> and give to baby tissue

### M. Sickle cell anemia

300 single site mutation of Hb known

one of them Hb S is responsible for sickle cell anemia

Glu→Val in position 6 of beta

deoxy HB has hydrophobic patch

Starts hemoglobin aggregating to form strand and crystals

Figure 5-20

This in turn makes cells sickle and clog capillaries

Figure 5-19

For heterozygotes not too bad live normal life if avoid vigorous exercise

For homozygotes can be fatal in childhood (or later if survive)

Why is deleterious gene in gene pool?

Heterozygotes have small but significant resistance to certain kinds of malaria!

## 5.2 Complementary Interaction between proteins and ligand

The immune system

A lot of good stuff, but I want to get on with proteins. It would advise the Premed to read the section on the immune system several times, it has lots of good information, but I must skip for now. Below are the set of notes I would use if I taught this section

Note: I have reorganized the material in the book in a way that makes better sense to me. I have tired mention in the notes when I have skipped around a section

**Immunoglobulins -antibodies-** key protein in immune system  
specifically designed to bind to other objects  
Binding to objects is key part of protein function so that is tie here

But also a biochemical understanding of the immune system is also an important subject in and of itself, so that is what part this section is about as well.

Lets start with this background

### A. Immune response - specialized array of cells and proteins

Two complementary systems

Humoral system - fluid system

Designed to remove infections (bacteria and viruses) from fluid around cells

Can also remove individual proteins that are in organism

Cellular immune system

Designed to kill host cells (the organisms own cells) that are infected with viruses. Can also kill some parasites. Will also be part system that recognizes and destroys foreign tissue in a transplant.

Keys cells - Leukocytes or white blood cells

**Macrophages** - job is to ingest large particles and cells by phagocytosis

**B lymphocytes** - key player in Humoral system

Developed from undifferentiated stem cell in bone marrow  
 Called B because last part of differentiation take place in the B or bone

Main job - produce Antibodies or Immunoglobulins (Ig's)

Soluble proteins that bind foreign material

Material can be bacteria, virus or large molecule

Make up 20% of all blood protein!

Soluble part means is release from cell and free in blood

**T lymphocytes** - key player in cellular immune system

Also develops from undifferentiated stem cell in bone marrow

But in this case final differentiation in the thymus

Hence the name T-cell for thymus

2 main types

cytotoxic T cells ( $T_C$  cells) or killer T cells

- Receptor protein (called T-cell receptor) on surface of T cell binds to object on surface of infected cell or parasite

- Bulk of receptor protein found on outer surface of cell, but extend all the way through the cell membrane and has a portion on the inside as well. (Will get to this in a chapter or two)

- When binds to object, structural change takes place on part of protein inside cell and this triggers changes in cell that start the killer response

Helper T cells - ( $T_H$  cells)

- Produces soluble signaling proteins - called cytokines

- This included interleukins

- This stimulates  $T_C$   $T_H$  and B cells to proliferate

Also interacts with macrophages

- So in general kicks all immune response up a notch for those cells that are needed to fight a particular infection

More terminology

**Antigen** - any molecule or pathogen capable of eliciting an immune response

- May be virus, bacterial cell wall, or other macromolecule
- Not small molecules (<5,000 MW)
- If want to make small molecule antigenic need to attach to large molecule

These large molecules are called **Haptens**

Large antigens can have many binding sites for different antibodies

- An individual antibody or T-cell receptor will bind at one particular molecular structure
- This structure is called in **Epitope** or an **antigenic determinate**

So how do you build a protein to bind a foreign object at a randomly chosen epitope??

(Note will discuss only structure of immunoglobins, book does not discuss structure of T-cell receptors in this chapter)

*B. Antibodies have two identical Antigen Binding sites*

Note: have temporarily skipped section Section on MHC.

Five classes of immunoglobulins or antibodies

IgA, IgD, IgG, IgE and IgM

IgG is most abundant so will start with him

Structure of IgG

- 4 peptide chains

2 large chains called heavy chains

2 smaller chains called light chains

See figure 5-21

- Linked together with disulfide and noncovalent interactions

- Make a 'Y'- shaped molecule total mass about 150,000

- 2 heavies interact with each other at one end

- Then a flexible hinge and each heavy interacts with a single light. This heavy/light section contains antibody binding site

- Base part of joined heavies may be cleaved and

separated from antibody binding region by proteases

-Base fragment alone called the **Fc** because readily

crystalized (Fragment, crystalizing)

- Heavy/Light section called **Fab** (Fragment, antigen-binding)

- Each Fab branch has a single antigen-binding site

- Heavy chain has three domains of relatively constant sequence (Designated  $C_H1$ ,  $C_H2$  and  $C_H3$ ), and one region with a highly variable sequence ( $V_H$ )

- The light chain has a single constant and a single variable region ( $C_L$  and  $V_L$ )

- Can see that these constant regions are characteristic beta-barrel domains with one part of barrel coming from one chain and the other  $\frac{1}{2}$  from the other chain

- Structure called the **Immunoglobulin fold**

- Antigen binding site is in variable regions
  - Makes sense want to make lots of different binding sites for lots of different antigens

- How do you make 10,000s of different proteins to bind 10,000 different antigen? Will save that secret for chapter 25)

Would think that would now examine structure of binding site bound to antigen, but to do that we have to skip ahead to:

### *C. Antibodies Bind tightly and specifically to Antigen*

- Binding specificity is determined by sequence of variable regions (light and heavy)

- In fact antigen binding site is hypervariable - more variable than rest of variable region

- Specific binding conferred by complementarity between antibody and antigen

- different interaction used

- Polarity, H bonding, charge, shape

- Binding site flexible, will change shape to optimize interaction with antigen

- Antigen may change structure to better fit into binding site (Will call Induced fit in next chapter)

See figure 5-25

- Very strong and specific binding
- $K_d = 10^{-10}$  M  
This means that will bind when concentration of antibody and antigen are as low as  $10^{-5}$ M (sqrt( $10^{-10}$ ))
- $K_d$  of  $10^{-10}$  mean that binding energy is on the order of 65 kJ/mole

Now back to general discussion of immunoglobulins

#### Functions of IgG

IgG is main antibody circulating in blood  
Major early player in 'primary' immune response  
First response to infection

- IgG is also major antibody in 'secondary' immune response
- Secondary response - initiated by memory B cells
- Response to an antibody that body has already dealt with at one time (I.E. already infected one, and now you should be immune because your body kept a memory of that antigen)

-First interaction is binding of antigen to antibody to tie up antigen

- But then there are other interactions as well

- When binds antigen activates other leukocytes like macrophages to engulf and destroy invader

-Macrophage has binding site for  $F_c$  Region of IgG

When binds IgG/antigen macrophage activates

**Figure 5-24**

- Also activates other parts of immune response

#### Differences in structure between different Ig's

Actual differences due to sequence of heavy chains

$\alpha$  - IgA

$\delta$  - IgD

$\epsilon$  - IgE

$\gamma$  - IgG

$\mu$  - IgM

2 types of light chains -  $\kappa$ ,  $\lambda$

Occur in all Ig's

Overall structure of D, E, and G are similar  
 M is either monomeric , membrane bound or crosslinked pentamer  
 (See figure 5-23)  
 A is monomer, dimer or trimer  
 Found in secretions like tears, saliva, milk

Early in immune response a B lymphocyte cell will make make IgG  
 Later will make IgD with same antigen binding site as IgG.  
 Function of IgD and reason for addition is not known

In Chapter 25 will learn how a cell with a finite amount of genetic code can make IgG's for essentially an infinite amount of different antigens. Through clever genetic recombinations your body can make antibodies to just about any particular antigen But this brings about a different problem. If your body can make an infinite number of different antibodies, how do you prevent it from making antibodies that will react with your own cells? And how do you fine tune the immune system to just make the antibodies you need to fight a particular infection, rather than making hundred of antibodies that don't react with anything?? To find that let's return to:

*D. Self distinguished from Non-self by the display of peptides on cell Surfaces*

This is a job another set of proteins called MHC

- Major Histocompatibility Complex

-Two Main types of MHC's

Class I MHC (Figure 5-21A)

Found on surface of almost all vertebrate cells

Each individual produces up to 6 variants

Set is unique for each individual

One  $\alpha$  chain that spans membrane and is highly variable

I would guess this is what binds the piece for display

One  $\beta$  chain that is invariant

Used to display bits and pieces of proteins degraded inside cell on surface.

Proteins degraded in cell include all proteins used in the cell

Part of proteins life cycle is to be made and degraded

But degrade proteins can include proteins of virus or bacteria that is attaching the cell

Each major class of MHC I protein is recognized by a specific receptor on a  $T_c$  (killer cell)

As  $T_c$  cells mature in thymus

Stringent selection process

Destroys 95 % of cells

Including one that recognize MHC I protein complexed with proteins that are normal part of cell

5% that are allowed to mature

Recognize MHC I bonded to foreign peptides

Thus only killer cells that recognize bad guys are allowed to mature and get released into blood

Note: how the cell tells the  $T_C$  cell that this is a normal or an abnormal peptide is not mentioned in text. I assume this is waiting for you to discover

MHC I proteins in transplanted tissue are recognized as foreign by bodies  $T_C$  cells and  $T_C$  deall bind, attach and this is beginning of tissue rejection

#### Class II MHC (Figure 5-21b)

Occur on surface of a few specialized cells

Includes Macrophages and B lymphocytes

2 membrane spanning peptides

Both have constant and variable regions

Like MHC I highly polymorphic - up to 12 variants per individual

So again a unique identifier for individual

Displays peptide that are derived from external proteins that have been digested by cell

So in case of macrophage displays pieces of cells it has been munching on, so that allows you to make additional killer sites to attack the attaching cell

MHC II recognized by both  $T_C$  (killer cells) and  $T_H$  (helper) cells

Use in both to filter the cells that are allowed to mature and run through the blood stream

#### General note of the life of a T cell

typical lifetime only a few days

So your body is continually making new cells and each cell binds a unique peptide epitope

Most cells never find anything to bind so die off

Only those cells that find something to bind to, trigger the immune response and get propogated

E. *The Antibody-Antigen Interaction is the Basis of a Variety of Analytical procedures*

Since antibodies are released into the blood, can make and isolate antibodies. For instance challenge a rabbit with a specific antigen, wait a few days, challenge again, do this several times, then extract remove a blood sample and isolate antibodies from blood.

Once isolate antibody now have a protein that is designed to bind to the antigen and can do nifty analytical techniques with this antibody.

Talk about two different types of antibody preps

Polyclonal antibodies - In normal immune response get many different B lymphocytes the recognize an antigen. Since each lymphocyte make and antibody for a different target site on the antigen, get a mixture of antibodies that bind in many different places on the antigen.

Monoclonal antibodies. First isolate a single lymphocyte and clone it. Now get only antibodies to a single specific epitope

Uses for antibodies

Attach to resin to make an affinity column

Make antibody radioactive or fluorescent

Then can be used to tag and identify antigen

Can be used to see where it is in a cell or in a gel

ELISA (Enzyme linked immunosorbant assay)

**Figure 5-26**

Say have 96 blood samples that you want to screen for Herpes

1. Put a drop of blood into 96 wells on a plastic dish  
(If cell has HERPES than a HERPES protein will adhere to plastic)
2. Wash of excess and put a drop of nonspecific protein into each well to cover up all possible protein binding sites
3. Wash of excess, incubate with antibody toi Herpes protein  
(Will bind to protein that is bound to dish)
4. Wash off excess now bind an antibody to the first antibody  
Called a secondary antibody  
This antibody has an enzyme covalently attached  
Enzyme will run a reaction that makes a reagent change color
5. Add reagent  
Only those wells that have Herpes bond to antibody bound to antibody bond to enzyme will change color

Immunoblot assay - used in electrophoresis

Transfer 1D or 2D electrophoresis gel to nitrocellulose to bind proteins

Treat membrane as we just did for ELISA

Will see colored bands where target protein occurs in gel.

5.3 Protein interactions of muscle

again lots of good stuff, but I will have to skip and move on