Proteins in action

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1 Introduction

The material in Nick Gay's lectures doesn't seem to really fit in with the rest of the course, so I've separated them into a different document. They are quite brief in style.

2 Antibodies

- Antibodies consist of two light chains and two heavy chains linked by disulphide bonds. They are produced by B-cells.
- Each B-cell produces a unique kind of antibody.
- In two dimensions they look like a Y with the inside of the arms of the Y made from the light chains and the outside of the arms and the stem made from the heavy chains.
- The N-terminal ends of all four chains are found at the tips of the arms of the Y and contain the variable regions which bind antigens.
- Normally the immune system throws everything at antigens, so many different antibodies are produced in response to a single antigen.
- In order to study their structure and develop therapeutics a method for getting a single immortal B-cell line was developed by fusing cancer cells with B cells and diluting down so that only single cells were looked at. This is called the **hybridoma** method.
- Initially mouse antibodies to cancerous cells were tried, but the antibodies themselves produced an immune response so the variable regions (called the complementarity determining regions or CDRs) were grafted onto human antibodies. These worked.
- The hybridoma method of developing monoclonal antibodies is time consuming and expensive, so the **phage display** method was developed, which fuses the cDNA of the CDR of a whole library of antibodies with the DNA of a bacteriophage in a coat protein gene. Those bacteriophages which "stick" to selected antigens already

contain the DNA encoding their CDR and so antibodies can easily be developed containing this CDR.

- Herceptin is a monoclonal antibody against the EGFR, which is over-expressed in 25–30% of breast cancers.
- The SLAM method is another method for increasing the efficiency of monoclonal antibody discovery.
- Enbrel and Humira are TNFα blocking drugs. Enbrel uses the TNF receptor as the arms of the Y of an antibody, while Humira is a monoclonal antibody. They both work amazingly well and have significantly improved the lives of many people with autoimmune diseases such as rheumatoid arthritis, though there is some indication that they leave the patient open to opportunistic infections, notably TB.

3 Proteases

- The two types of proteases we need to know about are **serine proteases** and **aspartic proteases**.
- Examples of the serine proteases are the pancreatic enzymes **trypsin**, **chymotrypsin** and **elastase** in addition to **thrombin**.
- Examples of aspartic proteases are the stomach enzymes **pepsin** and **rennin**, as well as **renin** and the **HIV1 protease**.
- In both cases, these proteases will only cleave peptide bonds between specific residues.
- Chymotrypsin only cleaves bonds following large hydrophobic residues[1].
- Trypsin only cleaves bonds after large positively charged residues.
- Elastase only cleaves bonds after small residues.
- The HIV1 protease is extremely specific, as appropriate cleavage points depend on the four residues before and the four residues after the bond.
- The reason HIV1 protease needs to be so specific is that the entire genome of HIV is translated into two polypeptide chains (*gag* and *gag-pol*), which must be cut at the correct locations.
- Three of the eight locations where HIV1 protease cuts are bonds which are not cut by human aspartic proteases, so drug companies have made synthetic inhibitors based on these structures in an attempt to block HIV infection.

[1]This is not quite true but it's what it says in the notes. For example it will quite happily cleave binds following tyrosine residues.

- Unfortunately this has proved difficult due to HIV's high mutation rate and the low bio-availability of many of the proposed synthetic chemicals. That said, combination therapy of protease inhibitors and reverse transcriptase inhibitors seems to be keeping viral load down and immune system function up.
- The big down-side of these drugs is that they are increadibly expensive and the majority of people with HIV live in poor countries.
- The method of action of these proteases is not that complicated if you break it down into little bits, it's just badly explained.

Mechanism of action of serine proteases

- Basically, the carbon of the scissile bond[2] is first bonded to the O of the OH group of serine 195 and the other end gets the H from that group and is released.
- A water molecule then comes along and gives its H to the remaining O of the serine 195's OH group, taking the carbon which was attached there in return.
- The purpose of the Aspartate 102 and Histidine 57 groups (the so-called **oxyanion hole**) is to stabilise these transfers, creating a **charge-relay system**.
- In the middle of each of these two steps a **tetrahedral intermediate** is formed, which is just a half-way house between the start and end points of the steps.
- The tetrahedral intermediate is the target for inhibitors, as it is the transition state and you may recall from enzyme kinetics that in an efficient enzyme the transition state is always more tightly bound than the substrates or products.

Mechanism of action of aspartic proteases

- Although the notes tell us that the mechanism of action of the HIV1 protease is as described, this does not in fact appear to be the case. The mechanism of action described is that of eukaryotic aspartic proteases like pepsin, which is slightly different from the HIV1 protease.
- The method of action of these proteases is actually simpler than that of serine proteases.
- Instead of actually binding to the enzyme, as happens with serine proteases, the role of the two aspartate residues at the heart of the protease is simply to stabilise the process of moving the OH from a water molecule onto the C of the peptide bond and the H onto the N.

[2] scissile means "easily cut". In this context the "scissile bond" is the peptide bond which the protease is going to cut

- This is achieved by starting with one of the aspartate's protonated and one not so that in the first step one can donate a proton to the substrate and the other can receive one from the water molecule. The fact that the activity of pepsin peaks around pH2 (the pK of aspartate) is a pretty good indicator that this is the case.
- This helps to form the tetrahedral intermediate of the transistion state.
- The picture in Voet and Voet 3rd ed. Figure 15-37 is pretty clear.

Other proteases

- Matrix metaloproteases are primarily involved with breaking down the basement membrane so that tumours can invade other tissues and stimulate angiogenesis to gain a blood supply.
- They have been targeted by biotech companies for cancer therapeutics but without much success.
- The metal ion basically acts to stabilise the transition state.
- Cysteine proteases act very much like serine proteases with the SH group of the cysteine acting like the OH group of the serine.