Michaelmas Practicals

Lysozyme

- Digests peptidoglycan cell walls (antibiotics just inhibit new formation)
- Tough - first to be identified by X-ray crystalography
- Gram-positive bacteria are much more sensitive to it
  - Because gram-negative bacteria have an out membrane that covers the peptidoglycan and makes it inaccessible to attack
- *Micrococcus luteus* is particularly sensitive
- Can use it to assay enzyme's activity
  - Bacteria scatter light
  - When their wall is digested, they burst and stop scattering light
  - Light transmitted increases
  - Causes a drop in "apparently absorbance" (attenuance)
  - Use **600 nm** (convenient but arbitrary)
  - Doesn't exactly follow Beer's law, but good enough
    - Mostly linear for low concs, though
- Solution of bacteria settles - must shake once in a while
- Use in phosphate buffer

Results

- **Just lysozyme**
  - Exponential decay
  - Maybe because number of walls left decreaes
  - Maybe because smaller bits of cell wall produced are still substrates for lysozyme, but digesting them doesn't cause too much of a drop in absorbance
  - Need to find the **initial rate**
- **Changing concentration**
  - Initial rate proportional to conc
  - Seems to saturate
    - Perhaps difficulties with assaying fast rate
    - Perhaps because enzyme is so small compared to substrate
- **pH**
  - About 6 is the best pH
  - Stryer notes that the carboxylic side chain of E35 should be undissociated, whereas that of D52 should be
  - pK values for these are 6.0 and 4.6
  - Stryer says pH optimum for chitting digestion is 5.0
- **Temperature**
  - 90 gets back for it, 80 loses about 1/2
  - Quite stable because small and four S-S bridges
  - Stability depends on environment - if heated in pH 6.4, OK
  - Heating with DDT completely kills it, because it reduces the S-S bridges

Denaturation

- Denaturation is a **cooperative process**
- Once it's started, it's easier to go on
- There are there abrupt changes
- SDS is a detergent - **binds to the protein and gives it a strong negative chargeb**
- Can't break covalent bonds - need a **reducing agent** for that. Use **DTT (dithiothreitol)**
  - Forms a stable ring when becomes oxidised
- Proteins in cells **do not** have **disulphide bridges**, because cells contain **thioredoxin**, which is a 1000 times more powerful reducing agent than DDT and is kept in a reduced form by an enzyme
- It is proteins **outside** cells, in unfriendly environments, that need the disulphide bridges

General stuff

- Duplicates help cancel out the effects of **random errors** and **variation**
- Controls (blanks) allow the substraction of bkacground readings from the experimental ones to obtain a true value
- 1 litre = 1000 ml = 1000 cm3

Absorbance

- Absorbance (A) = log (Incident light / Transmitted light)
- **Beer's Law** is **A = ecl**
  - **e** is the **absorbance coefficient**
  - **c** is the concentration
  - **l** is the path length
- Absorbance measured at a wavelength that is strongly absorbed by the solute
- Important to zero with solvent, because it might also absorb at that wavelength
- Can then work out concentration of solvent either using standard curve or known extinction coefficient

Pipetting

- With the P100, amounts display in MICROLITRES
- With the P1000, amounts display in TENS OF MICROLITRES

Identifying molecules

DNA

- At **260 nm**, an absorbance of **1.0** in a **1 cm** cuvette with **1 ml** of solution represents **50 microgram** of **double-stranded DNA**
- This is UV light, and why it's so bad for you

Glucose

- Glucose does not absorb significantly at 260 nm
- First, create a standard curve with standard concs
- Add **somogyi's alkaline copper reagent** and place in a **boiling water bath** for **exactly 15 minutes**
  - Aldehyde or keto group can reduce copper in alkaline conditions
- Cool down in ice for 15 minutes
- Add **Nelson's arsenomolybdate reagent** and **mix well**
  - Causes bubble to form - can alter readings
  - Tap pippete
- Find absorbance at **500 nm**, zeroed with water treated by Somogyi's and Nelson's