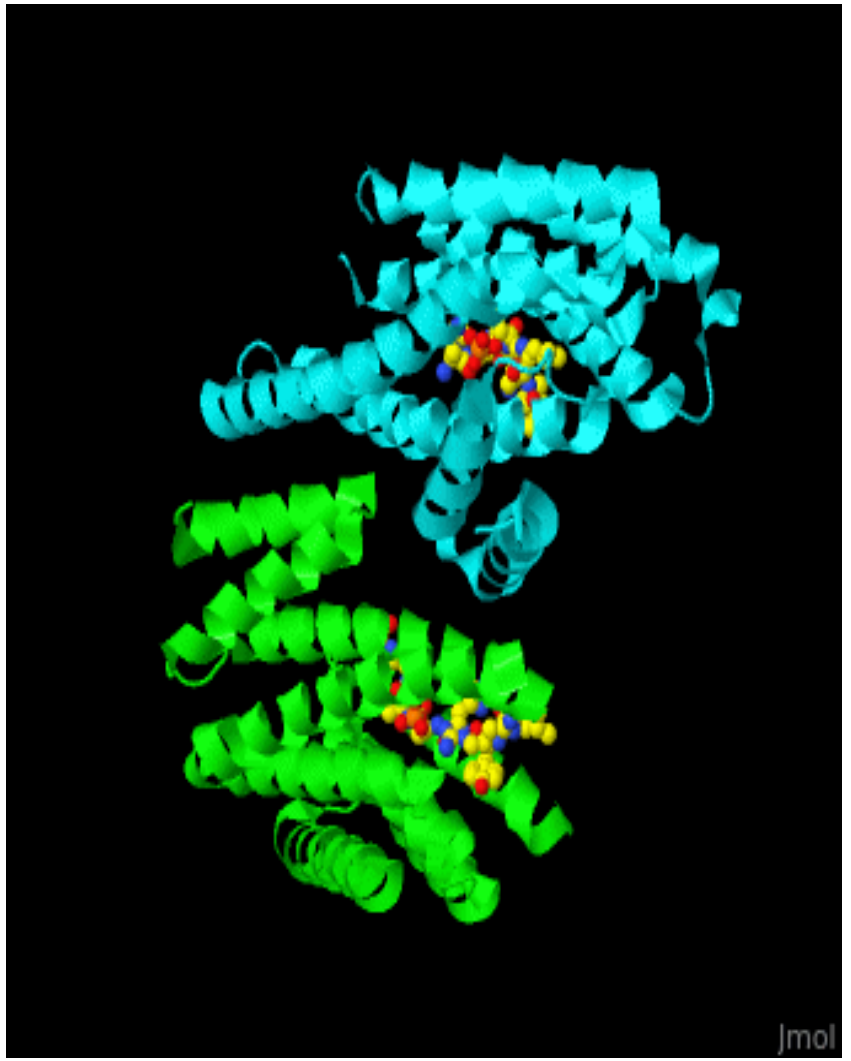
IIT Guwahati

Lecture 23

Course BT 631

Protein Structure, Function and Crystallography



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Enzyme Activity

"Enzyme unit" is defined as the amount of enzyme producing 1 µmole of product per min. 1U = 1 µmol/min

Enzyme Activity (EA) = 5.0 U/ml or 5.0 $\mu mol.min^{-1}.ml^{-1}$

Protein Concentration [P] = 0.5 mg/ml

Specific activity = EA/[P] = 5.0/0.5 = 10.0 U/mg or 10.0 $\mu mol.min^{-1}.mg^{-1}$

Specific activity of an enzyme is defined as the Amount of enzyme required per mg to produce 1 µmole of product per min.

Enzyme Assay

Product or Substrate

$$A + B \rightarrow C + D$$

Since the reaction is stoichiometric, in principle the rate may be measured equally well by monitoring the appearance of C or D or the disappearance of either A or B.

Depending on the method of measurement, there may be some advantage in opting for measurement of product formation, because it is easier to measure precisely a finite increase from zero than a small decrease from a large substrate concentration.

Physical or chemical property to measure

The choice will be governed by availability of equipment, cost of material and quality of the assay procedure.

Enzyme Assay Methods

Assay	Principle	Examples
Spectrophotometry (Absorbance)	Suitable when there is a difference between substrate and product in the absorbance of light of a particular wave-length according to the Beer-Lambert law.	Lipoxidase, dehydrogenase (NAD, 340 nm), kinase (ATP), Acetyl xylan esterase (pNP at 410 nm)
Spectrofluorimetry (Fluorescence)	Used when there is a difference in fluorescence between substrate and product. More sensitive than Spectrophotometry. Disadvantages: i) fluorescence varies with temperature; ii) quenching	Firefly luciferase, NAD(P) dependent dehydrogenases
Manometry (Pressure difference)	For reaction where there is gas uptake or output, and also those involving the production or consumption of acid or alkaline substances, if they are carried out in the presence of bicarbonate in the equilibrium with a gas mixture containing a defined percentage of CO_2.	Oxidases, decarboxylases
Electrode methods (Electrode potential)	For enzyme catalyzing redox reactions, Potential generated can either be measured by potentiometric techniques or related to the concentration of a specific substance, using ion-selective electrodes.	Lipase, carbonic anhydrase.

Enzyme Assay Methods

Assay	Principle	Examples
Microcalori-metry Heat (Enthalpy)	For reaction in which heat (enthalpy) is gained or lost.	Hexokinase
Radio-chemical labeling (Radioactivity)	By using radioactively labeled substrate, the product concentration can be determined indirectly by measuring the radioactivity of the product fraction. Extremely sensitive. Disadvantages: i) possible health hazard; ii) multi steps; iii) difficult to monitor reactions continuously; iv) quenching	Fatty acid synthetase
Polarimetry (Polarity)	For enzymes involved in conversion of optically inactive substrates to optically active products.	Lactate dehydrogenases (Lactate to Pyruvate)
Chromato-graphic methods	Chromatography can be used to detect the formation of a product when other methods fail. Disadvantage: time consuming	Chloramphenicol acetyl transferase

Enzyme Assay

Dependence on time and enzyme concentration

A basic assumption is an assay procedure is that doubling the enzyme addition will double the rate. However, in practice this can be true over a limited range of enzyme concentrations.

Addition of a large amount of enzyme could bring the assay reaction to equilibrium before observation even commenced. Therefore, a zero rate would be observed and the doubling the enzyme addition would make no difference.

Good linearity with the enzyme concentration can only be achieved if the reaction conditions are such that product formation is linear with time.

Enzyme Assay

Dependence on time and enzyme concentration

Frequently, a curved time-course is obtained so that attempting to measure the initial rate necessitates drawing a tangent.

Under these circumstances adding much less enzyme should result in a much-decreased extent of progress towards equilibrium and therefore more linear time course.

Ideally one should also aim to increase the sensitivity of observation;
for example, if a spectrophotometric assay trace is curved on the 0 - 0.5 absorbance scale, one could add 1/10 of enzyme and observe on the 0 - 0.05 absorbance scale.

Enzyme Assay

Dependence on time and enzyme concentration

Choosing the amount of enzyme to add. The upper curve shows a reaction trace where so much enzyme has been added that the reaction proceeds all the way to equilibrium during the observation period. Indeed, if observation does not commence promptly after initiating the reaction, the rise phase of the absorbance trace could be entirely missed.

The middle curve shows the same reaction with 10 times less enzyme added. This is still so curved that it is impossible to get a true initial rate by conventional manual mixing methods. Typically one might miss the 10-15 sec of the trace and then measure the tangent a false initial rate.

The lowest line shows the result of adding 10 times less enzyme than in the middle trace (i.e. 100 times less than in the upper trace). This slows down the reaction so that even in 60 sec the absorbance change is only 0.03 (approx.). This can however be made to extend over the full vertical scale simply by altering the range setting on the spectrophotometer to 0-0.05, as in the inset, instead of 0-0.5. The resulting trace is so close to linearity that the initial rate can be estimated accurately.