CONTROL OF PYRUVATE DEHYDROGENASE COMPLEX

(1) CONTROL OF PRODUCT INHIBITION: Acetyl CoA and NADH inhibit the enzyme complex and this is reversible by AMP.

(2) FEED BACK REGULATION BY NUCLEOTIDES: The enzyme is inhibited by GTP and AMP.

(3) REGULATION BY COVALENT MODIFICATION: The enzyme complex becomes enzymatically inactive when a specific serine residue of pyruvate dehydrogenase complex is phosphorylated by ATP.

MECHANISM OF ACTION: PYRUVATE CARBOXYLASE (I.e CATALYTIC ACTIVITY).

Pyruvate carboxylase catalyses the conversion of pyruvate to oxaloacetate. It has both catalytic and allosteric properties. It contain a prosthetic group (biotin) which serves as a carrier of activated co_2. this biotin is linked to the epsilon amino group of specific lysine residue by an amide bond. The carboxylation processes occur in two (2) stages thus:

(1) Biotin-energy + ATP + $HCO_3 \rightleftharpoons CO_2$-biotin-enz + ADP + pi

(2) Co_2-biotin-enz + pyruvate $\rightleftharpoons$ -biotin-enz + oxaloacetate

The carboxyl group in the carboxy-biotin enzyme intermediate is bounded to N-1 of biotin ring. The carboxyl group Is activated thus:

Co_2-biotin-enz + H $\rightleftharpoons$ CO_2 + biotin-enz

The activated carboxyl group is then tranfered from the carboxyl –biotin to pyruvate to form oxaloacetate. The long flexible chain enables this prosthetic group to rotate from one active site of the enzyme (the ATP- biocarbonate site) to other (the pyruvate site).

CONTROL OF PYRUVATE CARBOXYLASE

The activity of pyruvate carboxylase is dependent largely on the presence of acetyl CoA because biotin will not be carboxylated unless acetyl coA (or a closely related acyl CoA) is bound to the enzyme. A high level of acetyl CoA signals the need for more oxaloacetate. If there is surplus of ATP , oxaloacetate will be cosumed in gluconeogenesis. If there is deficiency of ATP, oxaloacetate will enter into TCA cycle upon condensing with acetyl CoA to form citrate.

ENZYME ASSAY TECHNIQUES

Assay simply means measurement of the enzymatic activity that is based on the determination of either the rate of formation of the product or the rate of utilization or disappearance of reactant or substrate under control conditions. Most assays are carried out at 30°C-37°C. Adequate buffering capacity is always being ensured. Apparatus used must be clean.

Analytical assays may be classified as (1) continous (2) discontinuous.

In order to standardize the report on enzyme activities, the commission of enzyme of the international union of biochemistry defined a standard unit i.e International unit (I.U) of enzyme as the amount of enzyme that catalysed the formation of 1 micro mole of product per minute under defined condition. The concentration of enzyme in an impure preparation is expressed in terms of units/ml while the specificity activity is expressed as units/mg protein.

In most cases, as the enzyme is being purified, its specific activity is expected to increase to maximum.

K_{cat}: is defined as the amount of enzyme that catalyze I mole of substrate per second.

Turnover number: is defined as the number of moles of substrate transformed per minute per second.

There exist different enzyme assay techniques, however, irrespective of the principle of the chosen method, the enzyme assay requires the use of excess substrate (zero order kinetics at least equal to $10K_m$) and an appropriate control is required. This control is in all respect the same as the test assay but lacks either the enzyme or substrate. Both the test and the control must be subjected to the same experimental condition.

TYPES OF ENZYME ASSAY TECHNIQUES

(1) VISIBLE AND UV SPECTROPHOTOMETRIC METHOD
(2) SPECTROFLUORIMETRY
(3) RADIOISOTOPE
(4) IMMUNOCHEMICAL METHOD
(5) MACRO CALORIMETRIC METHOD
(6) MANOMETRIC METHOD
(7) COUPLE ASSAY METHOD/TECHNIQUES

REFERENCES

CONTACT: Keith, W and John.W (1995) Practical Biochemistry: Principle and technique. 4th edition; Cambridge University Press, pp 209-216.

ENZYME PURIFICATION

Enzymes are purified by employing successive chemical or physical fractionation procedures**.** However, it should be noted that there is no fast and hard rule in respect of the protocol to be controlled. It is usually by a trial and error study based on a pilot study.

The utmost idea of each step is to get rid of the contaminants as possible and retain much desired enzyme. The efficiency of each step is given by

(1) The yield or recovery (i.e percentage of the total enzyme activity originally present that is retained.

(2) The purification factor (the factor by which the specific activity of the preparation has increased).

The preparation of the cell free extract may be prepared by several means depending on the nature of the starting material (tissue, or cell, or organism) and the size of the preparation. For instance, cell breakage method may be by autolysis freeze thaw, mechanical grinding and holistic homogenization e.t.c. the resulting homogenate is usually centrifuged to remove unbroken cells and large debris. At times, differential centrifugation for mitochondria, chloroplast, microsome, ribosome, or nucleic acids could be carried out.

The purity of the final preparation should be checked by several methods before one can conclude if the final preparation is homogenous. Some of the criteria are

(1) Homogenous enzyme preparation should elute from an ion-exchange or gel filtration column as a single symmetrical activity and protein peak with a constant specific activity throughout.

(2) Probably give a single bond (if not a dimer or polymer without artifacts or background noise).

REGULATION OF ENZYME ACTIVITY

In every metabolic pathway, the activity of at least one enzyme is subject to regulation so that the flux of material through the pathway can be controlled.

There are at least 4 means of control namely:

(1) Allosteric control

(2) Feedback inhibition

(3) Control by reversible covalent modification

(4) Control by irreversible covalent modification

ALLOSTERIC CONTROL: Some enzymes are reversibly inhibited or activated by the presence of metabolites that are not their substrate or product. These metabolites, if inhibitory are normally distant products of the pathway, thereby providing negative feedback for the activity of the pathway.

The enzymes controlled in this way usually have additional binding site other than the active or substrate binding site. The binding of inhibitor or activators at distant site from active site often brought about conformational changes in the enzyme molecule which may decrease or increase its catalytic activity. Thus, allosteric enzymes usually compose of subunits and use to have multiple interacting active centres and often shows sigmoidal graph of initial rate versus [S] and therefore do not obey Michaelis Menten kinetics.

FEEDBACK INHIBITION: This is a means by which biosynthetic pathways are regulated and involves the process whereby and products or near end products control the metabolic flux by inhibiting one or more of the enzyme at the early part of the pathway. Often maximum feedback inhibition is attained only by the combined actions of multiple end products.

Feedback inhibition could be (1) sequential (2) concerted (3)cumulative (4) co-operative

ILLUSTRATION

CONTROL BY REVERSIBLE COVALENT MODIFICATION:

This regulation is often the response to a signal coming from outside the cells such as response to hormone. In this case, the enzyme is itself the substrate of other enzymes. One of these modifies the enzyme making it active while the other reverses the modification thereby is inactive. A typical example is the control of glycogen phosphorylase through phosphorylation and dephosphorylation of specific serine residue. The enzyme exist in two forms i.e glycogen phosphorylase a (active) and glycogen phosphorylase b (inactive)

CONTROL BY IRREVERSIBLE COVALENT MODIFICATION

The best known example of the enzyme that exhibit /undergo this mechanism or modification is a protease enzyme named chymotrypsinogen. The enzyme, when reacted with diisopropyl phosphofluoridate becomes inactivated because of the reaction of serine-195 with chemical coupled with the formation of covalent linkage and elimination of HF. Also, activation of zymogen is another example of this type of modification. For instance, activation of trypsinogen by enteropeptidase will result in formation of trypsin, which also acts on chymotrypsinogen and converts its to chymotrypsin.

ILLUSTRATION