

Critically discuss the methods for the measurement of ALP isoenzymes

Introduction

Isoenzymes are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. They usually display different kinetic parameters e.g. different K_m values and have different regulatory properties. Isoenzymes permit the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage. Isoenzymes are isoforms of enzymes. In many cases, they are coded for by homologous genes that have diverged over time. Allozymes represent enzymes from different alleles of the same gene. Isoenzymes represent enzymes from different genes that process or catalyse the same reaction. Isozymes are usually the result of gene duplication and can also arise from polyploidisation or nucleic acid hybridization. Over evolutionary time, if the function of the new variant remains, then it is likely that one or the other will be lost as mutations accumulate a pseudogene.

APL is a Hydrolase that is responsible for removing phosphate groups from many types of molecules including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. In humans, alkaline phosphatase is present in all tissues but it is particularly concentrated in

- liver
- bile duct
- kidney
- bone
- Placenta

Humans and most other mammals contain the following alkaline phosphatase isozymes

- Intestinal
- Tissue non-specific (liver/bone/kidney)
- Placental (Regan isozyme)

The liver, bone and kidney enzymes are different forms of the same gene product with differences in post translational modifications. Gut and placental forms are isoenzymes.

The request for the measurement of ALP isoenzymes usually rests on the question of why the ALP activity is elevated and from which organ. With the usual use to determine if a rise is due to bone or liver isoenzyme. There is evidence to suggest that measurement of specific isoenzymes of little clinical utility especially with the availability of other tests of hepatic function e.g. GGT and the more widespread availability of scanning technologies for bone and liver. Therefore one of the major disadvantages of all methods for ALP isoenzyme determination is their lack of clinical utility.

In terms of the measurement of ALP isoenzymes a number of methodologies have been used. These include:

- Electrophoresis
- Inhibition Assay
- Immunoassay

Electrophoresis

Electrophoretic separation of ALP isoenzymes is the most widely used technique in the laboratories that still offer this analysis. Methods can be based on cellulose acetate or agarose solid supports and usually utilize either sample pre-treatment of lectins in the course of electrophoresis to bring about separation of the isoenzymes. Methods that utilize lectins or sample pretreatment usually offer higher resolution of the two major isoforms (Bone and Liver) and are thus able to fully distinguish between these two isoforms. Some methods are however unable to reliably distinguish between the placental

and bone isoforms and require extra sample processing to achieve this. Electrophoretic methods are labour intensive and time consuming and are thus relatively expensive. Some electrophoretic methods also require long standing specialist knowledge for interpretation and are therefore best suited for use in specialist centres. They can be used to determine if a number of isoenzyme activities are elevated and are able to detect all isoenzyme forms.

Inhibition or Inactivity Assays

Inhibition methodologies rely on the specific inhibition of an isoenzyme with measurement of ALP activity pre and post inhibition to determine which isoenzyme is responsible for the bulk of the activity. The inhibition can be by chemical or physical means (e.g. heat). These assays are relatively simple to perform and are therefore relatively cheap. These assays can be done in almost any laboratory. They can be difficult to interpret especially when activity levels are borderline or when isoenzymes are contributing equally to the rise in activity. They may show an inability to detect some isoenzymes and are generally considered the poorest performing of all isoenzyme methodologies.

Immunoassay

Immunoassays are generally used to specifically determine the amount of bone isoenzyme present. The measurement of BAP in this manner is usually restricted to the specific measurement of bone activity rather than to determine if total raised ALP is bone or liver. These assays have found utility in the monitoring of bone activity when patients are taking bisphosphonates or related therapies. BAP is a very specific product of osteoblasts and the circulating form has a relatively long half life. Compared to most other bone markers, BAP is unaffected by renal clearance and is relatively free of diurnal variation. The assays are sensitive, specific and reliable. They are generally more expensive than the inhibition/inactivity or electrophoretic assays but are suitable for the measurement of a large number of samples (for example in clinical trials etc).