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Procedural Determination of Porcine Intestinal Alkaline Phosphatase (ALP) Activity Kinetics in Classical Nutrition Research

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Abstract. The determination of porcine intestinal alkaline phosphatase (ALP) is very essential in biological and biomedical researches as it is a versatile enzyme that plays very important roles, especially in the liver/bile duct functions as well as in bone developments and turn over. Due to its versatile importance in biological studies it is imperative to clearly understand how ALP can be determined in biological tissues to better understand the biological implications in a study involving knowing its level in the tissue being investigated, such as that of porcine tissue or in DNA-related studies. This paper details the step-by-step procedures involved in measuring ALP using the western blotting procedure in a step-wise fashion that simply entails the followings: tissue preparation, gel electrophoresis, transfer, blotting and detection, respectively.

Key words: Porcine, Alkaline phosphatase, Procedures, Detection and Determination

Introduction

ALP is a very versatile biological molecule in determining liver/bile duct functions, including bone developments and turn overs. Therefore, laboratory attendants and researchers need to be very abreast with its procedural determination principles. In relating to the principles therefore, alkaline phosphatase is usually assayed by the methods of Bessey *et al*. (1946) and as further modified by Engstom (1964). In reference to these and other researchers, the first step required is to incubate tissue sample with a buffered reagent, in this case Mg^{2+} and secondly to stop the reaction by dilution with alkali and thirdly measure the intensity or amount of color developed according to the method of Rej (1977). Therefore, the principle is dependent on the measurement of the rate of hydrolysis of various phosphate esters under specified conditions of temperature and pH.

To this point therefore, the enzyme reaction can be measured successfully by the detection of *p*-nitrophenol yield using *p*-nitrophenyl phosphate as the substrate at an optimal pH of 10.5. The degree of *p*-nitrophenol yield can be determined by measuring its absorbance at 400 nm by the use of spectrophotometer. The substrate (*p*-nitrophenyl phosphate) is colorless but on splitting off the phosphate group, the yellow salt of *p*-nitrophenol is liberated (absorption maximum, 400 mμ) according to the principle developed by Bessey *et al.* (1946). Here, the substrate is the indicator of the amount or degree of splitting and therefore a measure of the degree of phosphate activity.

The reaction is usually stopped by adding 1 ml of 0.25 M NaOH solution. Due to the fact that intracellular acid phosphatase in the intestinal mucosa has a broad spectrum pH optimum of 2.0 *mM*, potassium fluoride is usually added to the assay media to inhibit its interference with the determination process of the enzyme (Hubscher & West, 1965). The use of *p*-nitrophenyl phosphate as substrate in the determination of alkaline phosphatase activity kinetics has been shown to be split 15% faster than phenyl phosphate, 2 to 3 times more rapidly than glycerol phosphate and 25 to 30 times faster than phenolphthalein phosphate (Hubscher & West, 1965). As a result of this rapid splitting coupled with the high chromogenicity of p-nitrophenol, it has been suggested that the reagent is well-suited for

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adaptation to both micro- and macro-procedures. Therefore, the objectives of this paper are to marshal out these processes in details in a literature based fashion for easy adoption by attendants and modern/new researchers.

Major Steps for Western Blotting in Determining ALP in Porcine Tissue

In general, western blotting is a method to detect a specific protein in a given sample of tissue homogenate or extract. Usually, it involves the uses of gel electrophoresis to separate native or denatured proteins in relation to the length of the polypeptide. The proteins are then transferred to a membrane where they are probed or detected using antibodies specific to the target protein. The steps-by-steps procedures involved in the entire process as outlined by the principles of (Bessey *et al.* 1946; Engstom, 1964; Hubscher & West, 1965) as well as other researchers as detailed below.

Tissue Preparation

Solid tissues are first broken down mechanically using a blender (for large samples) or using a homogenizer (for small samples). After this, the use of assorted detergents, such as salts and buffers are employed mainly to aid in the lysis of cells and to solubilize proteins. Protease and phosphates inhibitors are often added to prevent the digestion of the sample by its own enzymes (Bahr & Wilkinson, 1967). A combination of biochemical and mechanical techniques involving different types of filtration and centrifugation are then usually used to separate different cell compartments and organelles. After tissue preparation, the next step to be followed is gel electrophoresis.

Gel Electrophoresis

This step involves the separations of the proteins of the sample using gel electrophoresis. The separation of proteins can be by isoelectric point, molecular weight, electric charge or by a combination of these factors. However, the nature of the separation depends on the treatment of the sample and the nature of the gel (Walker & Pollard, 1971). To date, the most common types of gel electrophoresis employed are polyacrylamide gels and buffers loaded with sodium dodecyl sulfate. Samples are usually loaded into wells after which they are transferred.

Transfer

To make the proteins accessible to antibody detection, they are normally moved from within the gel onto a membrane made of nitrocellulose. The membrane is usually placed on top of the gel and then a stack of tissue papers is placed on top. The entire stack is then placed in a buffer solution which moves up the paper by capillary action bringing the proteins with it principally for blotting (Bretaudiere, 1977).

Blotting

For the fact that the membrane has been chosen for its ability to bind protein and since both antibodies and the target are proteins, cautions or steps must be taken to prevent interactions between the membrane and the antibody used for detection of the target protein. Here, the blocking of non-specific binding is achieved by placing the membrane in a diluted protein solution (usually Bovine serum albumin or non-fat dry milk) within a minute percentage of detergent, such as Tween 20. This technique eliminates false positives in the final product of the Western blot resulting in clearer results for ALP detection (Bretaudiere, 1977).

Detection

In the detection process, the membrane is 'probed' for the protein of interest with a modified antibody which is linked to a reporter enzyme which when exposed to, an appropriate substrate drives a colorimetric reaction and produces a color (Horne *et al.* 1968; Shephard & Peake, 1986). The intensity of color development is synonymous with the level of ALP concentrations in the tissue. For a variety of reasons, detection traditionally takes place as a two-step-wise processes even though there are now one-step detection methods available for certain applications. The two-step applications generally involve primary- and secondary- antibodies, respectively.

Conclusions

The step-by-step procedures involved in the determination of ALP in a tissue, such as porcine intestinal phosphatase activity kinetics were clearly marshalled out in this paper. These step-by-step procedures involved tissue preparation, gel electrophoresis, transfer, blotting and finally detection based on calorimetric and clorimetric principles.

References

Bahr, M. & Wilkinson, J. H. (1967). Urea as a selective inhibitor of human tissue alkaline phosphatases. *Clin. Chim Acta, 17*(3), 367-370.

Bessey, O. A., Lowry, O. H. & Brock, M. J. (1946). A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *J. Biol. Chem., 164*, 321-329.

Bretaudiere, J. P. (1977). Criteria for establishing a standardized method for determining alkaline phosphatase activity. *Clin. Chem., 23*(12), 2263-74.

Engstom, L. (1964). Studies on bovine-liver alkaline phosphatase, purification, phosphate incorporation. *Biochimica et Biophysica Acta, 92*, 71-78.

Horne, M., Cornish, C. J. & Posen, S. (1968). Use of urea denaturation in the identification of human alkaline phosphatases. *J. lab. Clin. Med., 72*(6), 905-915.

Hubscher, G. & West, G. R. (1965). Specific assays of some phosphatases in subcellular fractions of small intestinal mucosa. *Nature, 205*, 799-800.

Rej, R. (1977). Effect of incubation with Mg2+ on the measurement of alkaline phosphatase activity. *Clin. Chem., 23*(10), 1903-1911.

Shephard, M. D. & Peake, M. J. (1986). Quantitative method for determining serum alkaline isoenzyme activity. *J. Clin. Pathol., 39*(9), 1025-1030.

Walker, A. W. & Pollard, A. C. (1971). Observation on serum alkaline phosphatase electrophoretic patterns on polyacrylamide gel with particular reference to the effects of butanol extraction. *Clin. Chim. Acta., 34*(1), 19-29.