Journal of Biological Sciences and Bioconservation
Volume 6, Number 1, 2014
ISSN: 2277-0143

TIME COURSE KINETICS OF ACID PHOSPHATASE AND β-GALACTOSIDASE OF STARVED *E. COLI* CELLS

H. Zanna[1], M.A. Milala[1], A.J. Nok[2], P. Wuyep[3] and E. Amlabu[2]
[1]Department of Biochemistry, University of Maiduguri, Maiduguri, Nigeria.
[2]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.
E-mail: mohammedmilala@yahoo.com.

ABSTRACT
The time course for the production of acid phosphatase and β-galactosidase in starved *E. coli* was investigated. *E. coli* cells were starved by growing the cells in basic medium containing 2% glycerol. β-galactosidase production was induced by 0.002M lactose. Acid phosphatase and β-galactosidase activities were assayed using PNPP and ONPG respectively. Time course kinetics was monitored every hour for nine hours and solid $(NH_4)_2SO_4$ was used to precipitate the two enzymes at 30-55% and 20-35% respectively. SDS-PAGE of the crude and $(NH_4)_2SO_4$ of partially purified enzymes was also carried out. Results revealed no correlation in terms of the time course kinetics between the two enzymes. However, the two enzymes belong to the same group of proteins that can be precipitated by $(NH_4)_2SO_4$.

Keywords: Acid Phosphatase, β-galactosidase, Time Course.

INTRODUCTION
Acid phosphatase (E.C. 3.1.3.2) belongs to a group of enzymes that hydrolyze phosphoric acid monoesters into a phosphate ion and a molecule with a free hydroxyl group. Protein phosphorylation and dephosphorylation is an important phenomenon in cellular metabolism (Bingham and Garver 1990). The action of phosphatases is directly opposite to that of phosphorylases and kinases, which attach phosphate groups to their substrates by using energetic molecules like ATP. Phosphatases are classified into two: cysteine-dependent phosphatases and metallo-phosphatases (Barford, 1996). Phosphatases are integral to many signal transduction pathway because addition of a phosphate group may activate or

deactivate an enzyme or enable a protein-protein interaction to occur (Ladbury 2007). The study of acid phosphatase is difficult because of their multiform occurrence in organisms, relative – non specificity, small quantity and instability in dilute solution (Tso and Chen, 2007).

The enzymatic hydrolysis of lactose to glucose and galactose with β-galactosidase is one of the most ancient biotechnological processes known. The microorganisms usually associated with β-galactosidase high production is the *Escherichia coli, Bacillus megaterium*. *E. coli* β-galactosidase is an inducible enzyme that is synthesized in the cell only when an inducer or signal is present in the cell. Cells that are deficient in lactose do not synthesize β-galactosidase, but in a medium containing lactose, synthesis of β-galactosidase is done within minutes, enabling the cells to use lactose as a carbon source and energy for growth (Martin *et al;* 2000).

The improvement of β-galactosidase downstream processing from its microbial source would be desirable to reduce the cost of enzyme preparation, since extraction, purification and concentration steps are commonly responsible for up to 40% of total production cost (Kula, 1990).

The ability of β-galactosidase to hydrolyze lactose into galactose and glucose is applied in food and particularly in the field of dairy products because of nutritional (lactose intolerance), technological (crystallization) and environmental (pollution) problems associated with lactose (Triveni, 1975).

This study is aimed at examining the relationship between the time course for the production of acid phosphatase and β-galactosidase in starved *E. coli* cells.

MATERIALS AND METHODS

Materials

P-nitrophenyl phosphate (PNPP) and O. Nitrophenyl-β-galactoside ONPG were purchased from Sigma Chemical Company, St. Louis, U.S.A. *E. coli* cells were obtained from the culture stock of Microbiology Department, Ahmadu Bello University, Zaria, Nigeria and Mac Conkey broth was from Fluka Chemical Company.

Growth of Starved E. coli cells

E. coli cells were inoculated into 5ml Mac-Conkey broth containing 2% glycerol and shaken overnight at 37^0C in an incubator shaker at 200rpm. 2.5ml of the overnight culture was inoculated into 50ml of the MacConkey broth containing 2% glycerol. This made the cells now to be in the log phase of growth (Martin, 2000).

Induction of Enzyme

β-galactosidase is an inducible enzyme, its production was induced by mixing 4ml of starved *E. coli* cells and 0.2ml of 0.002M lactose and shaken for 30minutes.

Time Course Study

Time course for the production of acid phosphatase and β-galactosidase were monitored every hour for 9 hours.

Ammonium Phosphate Precipitation

Acid phosphatase: 20ml *E. coli* culture was precipitated at 30% and allowed to stand for sometimes. Sample was centrifuged at 10,000xg for 20min. the pellet discarded and more ammonium sulphate was added to the supernatant to obtain a concentration of 55%. The mixture then centrifuged for 20min at 10,000xg.

The pellets were dissolved in 50ml homogenation solution and dialysed overnight against a 50mM acetate buffer pH 5.2 with three changes. The dialysate was electrophoretically analyzed with 12% SDS-PAGE.

β-galactosidase: β-galactosidase was precipitated at 20-35% as described above using 0.1M phosphate buffer pH 7.2.

SDS-PAGE

This was carried out according to the method described by Laemmli (1970).

Acid Phosphatase Assay

Acid phosphatase activity was assayed in a 125μl reaction mixture containing 20mM PNPP and 50mM sodium acetate, PH 5.5 at 37^0C. Reactions were initiated with the addition of enzyme and terminated by addition of 750μl of 0.1M NaOH. The amount of P-nitrophenyl released was calculated using molar extinction coefficient

of 17,500$M^{-1}cm^{-1}$ at 400nm.one unit of enzyme activity is defined as the amount of enzyme that releases 1μmol P-nitrophenol per minute under assay condition.

β-Galactosidase Assay

β-galactosidase activity was assayed in a 1050μl reaction mixture containing 10mM ONPG and 100mM phosphate buffer pH 7.2 at 37^0C. Reactions were initiated with the addition of enzyme and terminated by addition of 125μl of 2M $NaC0_3$. The amount of ONP released was calculated using molar extinction coefficient of 13,700$M^{-1}cm^{-1}$ at 420nm. One unit of enzyme activity is defined as the amount of enzyme that releases 1μmol ONP per minute under assay conditions.

Protein Determination

Proteins were estimated by Biuret method (Henry *et al.,* 1974). Bovine serum albumin was used as the standard.

RESULTS AND DISCUSSION

Fig. 1 and 2 showed the time course kinetics for the production of acid phosphatase and β-galactosidase. Production rate for acid phosphatase was highest after 2hours of inoculation. Enzyme activity dropped and started picking up at the seventh hour. While the production rate for β-galactosidase attained its peak after 8 hours well into the stationary phase. Dassa *et al.,* 1982 reported a premature full induction of acid phosphatase production in *E. coli* cells starved of inorganic phosphate while synthesis of the enzyme was turned off in exponentially growing bacteria and started as soon as cultures entered the stationary phase.

A summary of purification procedure is given in table 1, enzymes were purified by precipitation with $(NH_4)_2SO_4$. Fig 3 and 4 showed the 12% SDS-PAGE of the crude and $(NH_4)_2SO_4$ partially purified enzyme. The SDS-PAGE for the crude and $(NH4)_2SO_4$ precipitate of acid phosphatase and β-galactosidase showed double and single bands respectively. This might suggest that the enzymes could be in the same group of proteins that can be precipitated by $(NH_4)_2SO_4$. Overall, results suggest little or no correlation between the two enzymes. However, this is a preliminary study.

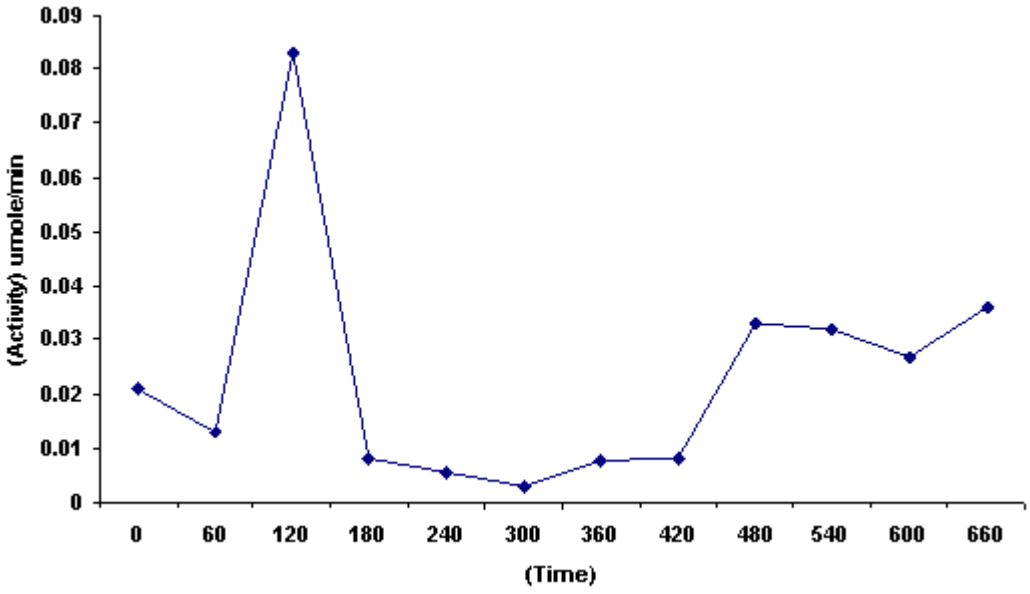

Figure 1: Time Course of Acid Phosphatase Production

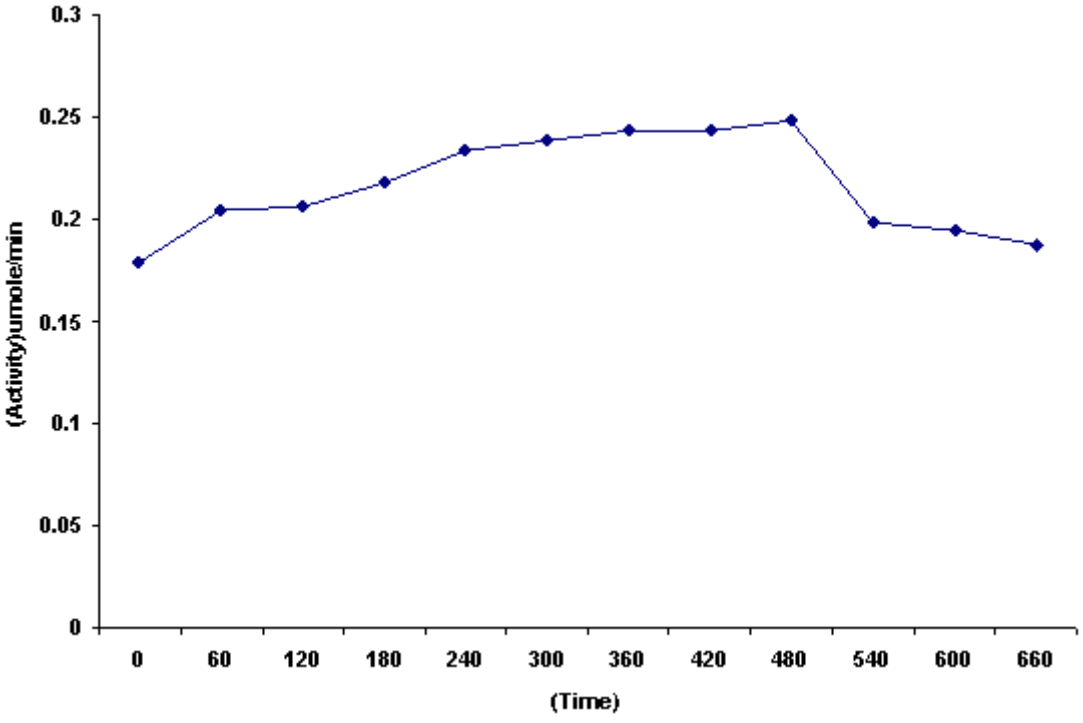

Figure 2: Time Course of β-Galactosidase Production

Table 1: $(NH_4)_2SO4$ Purification of *E. Coli* Acid Phosphatase and β-Galactosidase

Enzyme	Fraction	Volume (ml)	Protein (mg/ml)	Total Protein (mg)	Activity μmole/min	Specific Activity μmole/min/mg Protein	Purification Fold	% Yield
Acid Phosphatase	Crude	52.5	4.84	254.1	0.083	0.017	1	100
	$(NH_4)_2SO4$ 30-55%	20	2.13	42.6	0.02	0.094	0.55	24.1
β-Galactosidase	Crude	52.5	0.344	18.06	0.248	0.721	1	100
	$(NH_4)_2SO4$ 20-35%	20	0.231	4.6	0.22	0.954	1.32	88

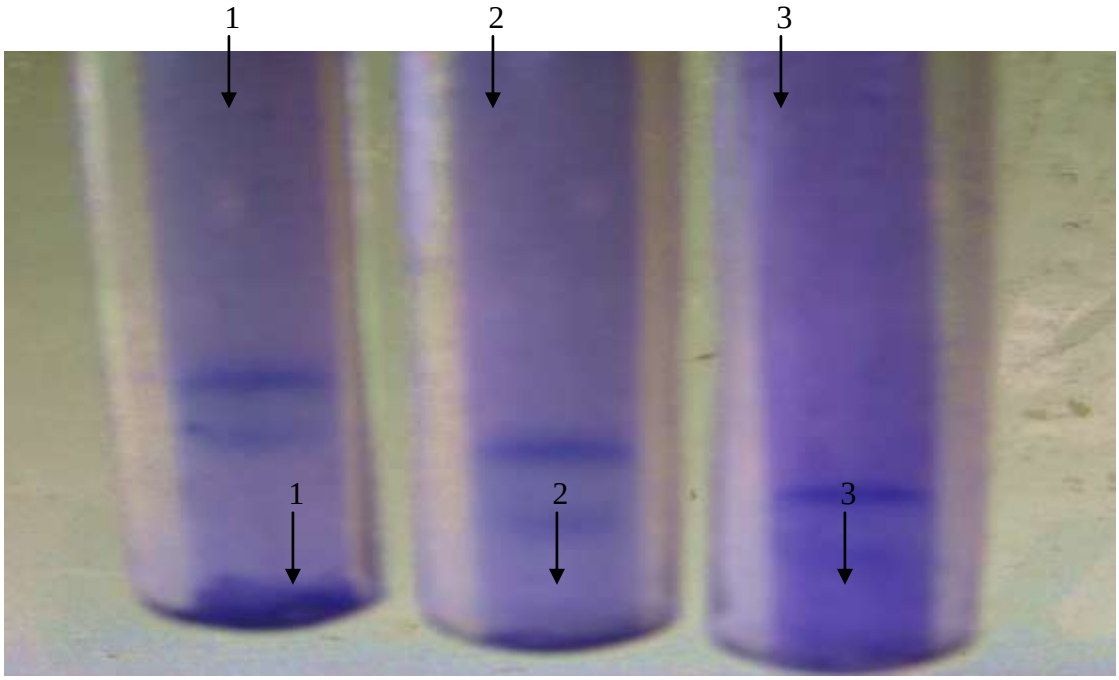

Fig. 3: 12% SDS-PAGE Electrophoregram of Crude Acid Phosphatase Solution (1 & 2) and $(NH_4)_2SO_4$ Purified Enzyme (3)

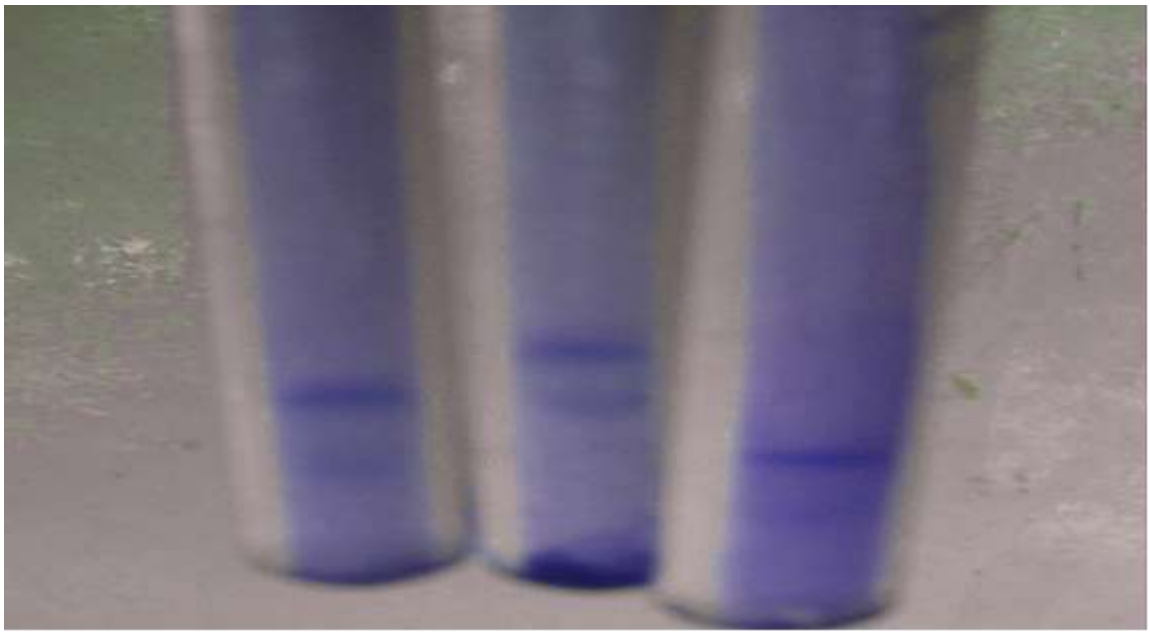

Fig. 4: 12% SDS-PAGE Electrophoregram of Crude β-Galactosidase Solution (1 & 2) and $(NH_4)_2SO_4$ Purified Enzyme (3)

REFERENCES

Barford, D. (1996). Molecular Mechanisms of the Protein Serine/Threonine Phosphatases. *Trends Bioch. Sci.* 21, 11 p. 407.

Bingham EW and Garver, K (1990). Purification and Properties of an Acid Phosphatase from Lactating Bovine Mammary Gland. *J. Diary Sci.* 73, 964-969.

Dassa; E., Cahu M., Desjoyaux – Cherel, B. and Boquet, P.L. (1982). The Acid Phosphatase with Optimum pH 2.5 of *Escherichia coli,* Physiological and Biochemical Study. *J. Biol. Chem. Vol.* 257 Issue 12 6669-6676.

Henry R., Cannon D.C, Winkelman J.W. (1974). Clinical Chemistry; Principles and Techniques, Herper and Roe, Maryland, USA. P. 543.

Kula; M.R. (1990). Trends and Future of Aqueous Two-Phase Extraction. *Bioseparation* 1:181-189.

Ladbury; J.E. (2007). Measurement of the Formation of Complexes in Tyrosine Kinase-Mediated Signal Transduction, Acta Cryst D, 62:26.

Laemmli, UK (1970). Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4. *Nature* 227:680-685.

Marrakchi M, Dzyadevych SV, Lagarde F, Martelet C, Jaffrezic-Renault N. (2008). Conductometric Biosensor Based on Glucose Oxidase and Beta-galactosidase for Specific Lactose Determination in Milk. Material Science and Engineering C. 28:872-875.

Martin-Vasallo J; Pressley P; Frank R.H, Muller-Ehmsen K, Xiang T.A. (2000). Photoreversible Modulators of *E. coli.* Beta-galactosidase. *J. Protein Chem.* 19(2) 123-128.

Triveni P.S. (1975). CRC Critical Reviews in Food Technology, 324-354.

Tso, Sc and Chen YR (1997). Isolation and Characterization of a Group III Isozyme of Acid Phosphatase from Rice Plants. *Bot. Bull. Acad. Sin* 38. 245-250.

Reference to this paper should be made as follows: M.A. Milala *et al.* (2014), Time Course Kinetics of Acid Phosphatase and β-galactosidase of Starved *E. coli* Cells. *J. of Biological Science and Bioconservation,* Vol. 6, No. 1, Pp. 21 – 28.