A kinetic Study of the Rate Reaction of Alkaline Phosphatase in Tuberculosis

H. G. Hassan and P. A. Ismahil
Department Chemistry, College of Science, University of Sulaimani
Department Chemistry, College of Education, University of Salahaddin

Abstract

Serum alkaline phosphatase activity has been investigated in three major types of tuberculosis (TB). These were *Extra pulmonary primary*, *Acute miliary*, and *Primary* tuberculosis. Activities were found elevated in these cases studied at basic PH and 37 C. The study concentrated an a comprehensive determination of the rate reaction kinetics of the enzyme reaction in both normal and TB sera. The pseudo first – order plot reflects both values of the first – order association constant (K1) and the half life time (t1/2) of the enzymatic reaction. The activation energy of the reaction (ES-complex formation) with Hill coefficient (n) were both estimated using Arrhenius and Hill plots respectively.

Introduction

Enzymes represent the major components of the biological fluids. They are maintained life continuity throughout their catalysis reactions. Alkaline phosphatase (AP) is highly ubiquitous enzyme, is a membrane-bound that is associated with virtually all cells. In humans, AP is encoded by a multi-gene family composed of four loci; i.e. , tissue-nonspecific AP also called bone-liver-kidney AP (1,2). Alkaline phosphatase in the lung has been localized to the plasma membrane and lamellar bodies of type II alveolar epithelial cells and its concentration in lung fluids has been used as a marker of damage of these cells (3). Serial estimations of AP activity provide of value in the follow-up of a patient with TB (4-6).

Adequate information on the kinetic changes of the biochemical reaction of enzymes is scarce (7). A comprehensive picture of the rate reaction kinetics is in need of an accurate determination of these parameters. The authors, studied heat activation as a concept not possessing more than a certain amount of kinetic energy are said to be activated. In enzyme reaction, activation of the substrate, occurs by the formation of the complex enzyme-substrate (ES) and the process has much in common with the formation of the activated complex of the absolute rate theory (8). In order to obtain cooperative binding of S, the enzyme must clearly have more than one binding site (8). A number of models were adopted to cooperative binding, one of these models is that considered by AV Hill in 1949, in which Hill coefficient (n) would correspond to the number of binding sites and equal to one so that a reaction would describe by a rectangular hyperbola.

In this paper, the authors have carried out a typical determination of the rate reaction kinetics in normal and TB for their reaction catalyzes by AP enzyme.

Materials and Methods

Chemicals: These were chosen for specific AP activity determination and all other experiments related. They were of analytical grade, and are highly purified, purchased from BDH and Riedel-Dehaen firms.

Blood collection and sera separation : These were of 30 normal healthy adults in addition to 70 samples represent TB individuals (37 males and 33 females), aged between 21-67 years, from where blood samples were collected. The TB samples consists of 37 *Extra pulmonary primary*, 25 *Acute military*, and 8 *Primary* cases. They were diagnosed over duration of 2-to-16 months periods, and they were under chemotherapy treatment. Normal and TB blood samples (5-10 mls) were aspirated using veinpuncture, left at room temperature for half an hour then spinning at 3000 rpm for 15 min for the appropriate sera.

Assay of AP activity : A protocol of King and King (9) was adopted for the determination of AP activity. The principle depends on phenolic group releases by enzymatic hydrolysis of phenol phosphate, as substrate, under defined condition of time, temp, and alkaline PH.

The activity was determined by using spectrometric analysis in which a colored complex is absorbed at 510nm, using series concentrations of the substrate [S] fig.(1), table (1).

Determination of the rate association constant K1 and the t1/2:
Following time course reaction, the order of the enzymatic reaction, its K1 and t1/2 were determined. The same protocol of activity was applied at constant [S] but the time of incubation was varied fig.(3, 4).

Determination of the activation energy Ea :
The same protocol for AP activity determination was adopted and the only difference is the use of various incubation temperature fig.(5).

Results

Follow-up the activities of AP in the 70 TB samples , it was seen that an elevation occurred in all three types of TB cases (156.203±21IU/L, 206.4±15 IU/L, and 135.3±35 IU/L respectively) in comparison with that of normal individuals (32.55±25 U/L). A hyperbolic relationship was obtained fig.(2) and shows a 2.7-fold increases in TB greater than in normal. Investigating the rate reaction kinetic mechanism and type of the order of the reaction, a pseudo first-order type fig.(3) were obtained in both normal and TB cases. The value of K1 and t1/2 table (2) was also obtained using the relation :

Ln (Vmax /Vmax –v) Vs T T – time .

From the above, the value of K1 is determined and the value of t1/2 was calculated as :

$$t1/2 = \frac{0.693}{K1}$$

Discussing Hill plot fig.(4), results show a value of Hill coefficient (n) to be about equally for both normal and TB enzymatic reactions table (3). Also, Arrehnius relationship fig.(5) revealed a higher level in the energy of activation in TB reaction than that in normal table(4).

Discussion

The determination of serum levels of AP activity and the tissular origin of increased amounts has clinical significance in the follow-up of

patients with a variety of afflictions (10-12), and was shown to be a remarkable marker in the diagnosis.

Investigating AP enzyme, literatures concentrated on its activity determination and few research were published concerning rate reaction kinetics determination (7). In TB disorder, studying AP activity revealed elevation in the enzyme activity in children (3). Mesjidi *et al.*, (6), found high levels of AP activity in both serum and pleural fluid in malignant and empyema groups of TB.

In our study, an elevation in AP activity was also seen in all the three cases of TB studied. These were found to be 2.7-fold greater than in normal individuals. One can conclude that as AP in the lung was lacalized to the plasma membrane, and these cells were damaged during their infection with TB, therefore, this could cause effusion of the cell fluids so that the AP enzyme levels will secretes down the circulation. Another choice of suggestion is that as the duration of infection became so long, this could lead to the damage of other specific tissues like bone resulting in increasing the level of AP in circulation (3,13).

Investigating the type of reaction order of AP reaction fig.(3), the data show that AP reaction in both normal and TB obey linearity and the straight line obtained indicate that the reaction is a first-order reaction. The slope obtained shows a value of K1 table (2), and the t1/2 value was estimated from the equation :

$$t1/2 = \text{In } 2 / K1 = \frac{0.693}{K1} \qquad (12,14)$$

It was seen that K1 value is higher in TB than that in normal, therefore, we can conclude that the disorder has its own effects in the equilibrium constant (Keq) of AP reaction and then on ES-complex formation throughout increasing the association rate (15,16) :

$$E + S \underset{k-1}{\overset{k1}{\rightleftharpoons}} ES$$

$$K\text{-}1\,[ES] = K1\,[E]\,[S]$$

$$Keq = K\text{-}1 / K1 = [E][S] / [ES]$$

The results of table (2), also showed decrease in t1/2 value in TB than in normal upon which the authors can suggest to be due to the acceleration in the enzyme activity because of the suggested damage occured in lung cells (3). In this situation, a high secretion of AP levels means increasing the binding affinity of the enzyme to its substrate, this can be explained to be due to the increase in the isoforms of the enzyme, thus, the number of the binding sites will increase (8). Hill's plot fig.(4) investigation shows a value of (n) to be equally (~ 1) in both the TB and normal and it was calculated (17) as :

$$\text{Log } [v/ Vmax - v] = n \text{ Log } [S] - \text{Log } K1.$$

Studying the energy of activation level for AP enzymatic reaction table (4) a value of Ea was estimated from Arrhenius plot fig.(5), in which Ea value found to be higher in TB than in normal. This can be explained to be due to the highly affected mechanism of the reaction in TB. Since the activation energy in TB is about 1.5-fold greater, therefore, AP in TB must obtain a greater amount of energy in order to overcome the energy barrier of the reaction to produce the product. This can be explained practically that the AP enzyme concentration increases in the circulation due to the releasing of the enzyme from site sources such as lung (3) and others. Confirming this suggestion, the value of H obtained:

$$H = Ea - RT$$

It was found that H value was positive (H = 9860.2 cal / mol.) and it was greater than that of normal (H =6129.1 ca l / mol.), therefore, the authors concluded that the study indicates that AP reaction is endothermic and the heat content of the activated complex (ES) is greater than that of the isolated species, i.e. E and S (18,19). This value of Ea was calculated from the slope of fig. (5) obtained in which :

$$\text{Log } v = - Ea / 2.3 RT + \text{Log } A$$

Refrences

1. Weiss, M.J.; Ray, K.; Henthron, P.S.; Lamb, B.; Kadesch, T. and Harris, H.J. (1988) Biol. Chem., 263: 12002-12010.
2. Henthorn, P.S.; Raducha, M.; Kadesch, T.; Weiss, M.J. and Harris, H. (1988) J. Biol. Chem., 263: 12011-12019.

3. Merino, J.M.; Curpintero, I.; Alvarez, T.; Rodigo, J.; Sandez, J. and Coello, J.M. (1999) Chest, 115: 26-30.
4. Waagner, C. (1993) Pediatr. Ann., 22: 622-628
5. Agrons, G.A.; Markowitz, R.I. and Kramer, S.S. (1993) Semin. Roentgenol., 2: 158-172.
6. Mesjedi, M.R.; Dokoohi, P.; Cheraghvandi, A.; Fadoi-Zadeh, L. and Mehdavi, S. (1998) webmaster @ sbmu.ac.ir.
7. Al-Mudhaffar, S.A.; Nasser, F. and Hassan, H.G. (1994) Iraqi J. Sci., 35(4): 1093-1106.
8. Dixon, M. and Webb, E., Enzymes 3rd.Ed. Academic Press, NY, London, PP5,169.
9. King, R.P.N. and King, E.J. (1954) J. Clin. Path., 7: 332-336.
10. Farley, J.M. (1988) J. Biol. Chem. 225: 4680.
11. Rwanduzy, G.H.A.(2001) An MSc thesis, College of Science, Univ. Sulaimani,supervised by Hassan HG.
12. Aziz, R.S. (2003) An MSc thesis , College of medicine, Univ. Salahaddin, Erbil.
13. Behnke, B.; Kemper, M.; Kruse, H.P. and Muller-Wiefel, D.E. (1998) Nephrol-Dial-Transplant, 13(3) : 662-667.
14. Fields, J.Z.; Roeskc, W.R.; Mordin, E. and Yamamura, H.I.(1987) J. B. Chem., 253: 3251-3258.
15. Ross, E.M.(1966) J. Biol. Chem., 252: 5761- 5775.
16. Minneman, K.P.; Weiland, G.A. and Molino, H.P.B. (1979). Molecular pharmacology, 17: 1-7.
17. Segal, I.H. Biochemical calculations 2nd.Ed. John Wiley and Sons, Inc. Printed in USA.
18. Marwa, U. and Hartshorne, D.L.(1980) Fed. Proc. Fed. Am. Soc. Exp. Biol., 39: 1564-1566 .
19. Plowman, K.M. Enzyme kinetics" 3rd.Ed. McGraw – Hill, NY. USA.

Table (1) Levels of AP- activities in 3-types TB disorder and normal Sera

Cases	AP- activity (Mean ± SD) IU / L
Extra pulmonary (EP)	156.203 ± 21
Acute miliary (AM)	206.4 ± 15
Primary (P)	135.3 ± 35
Normal (N)	32.55 ± 25

Table (2) Enzyme reaction parameters in both Normal & Tuberculosis

Enzyme	K_1 (min^{-1})		t1/2	
	Normal	Tuberculosis	Normal	Tuberculosis
APL	0.011	0.014	63	49

Table (3) Hill Cofficient (n) for S.AP in both Normal & Tuberculosis

Enzyme	Hill Cofficient (n)	
	Normal	Tuberculosis
AP	1.03	0.98

Table (4) Activation energy (Ea) for S.AP in both Normal & Tuberculosis

Enzyme	Activation energy (Ea)	
	Normal	Tuberculosis
AP	6739.9	10474

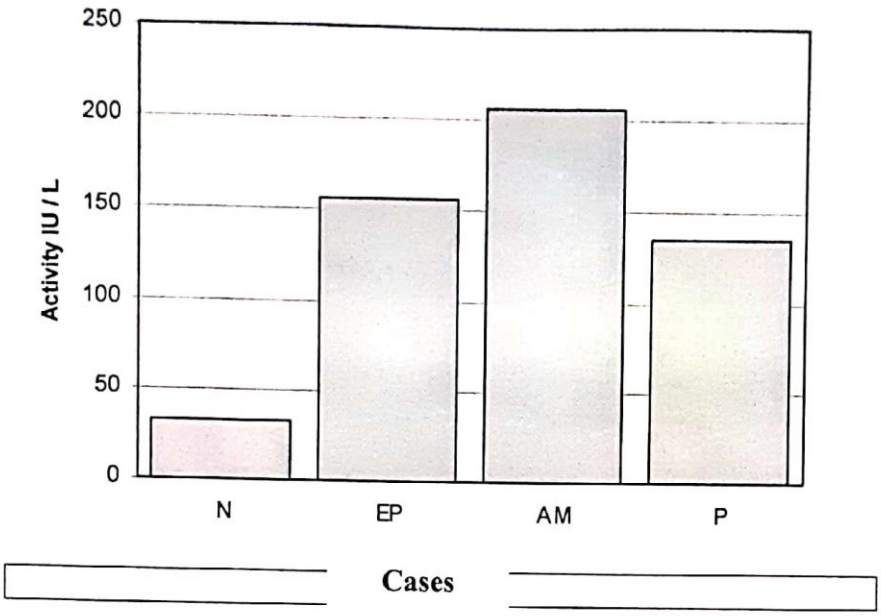

Fig. (1) Comparing AM activity in TB and normal sera

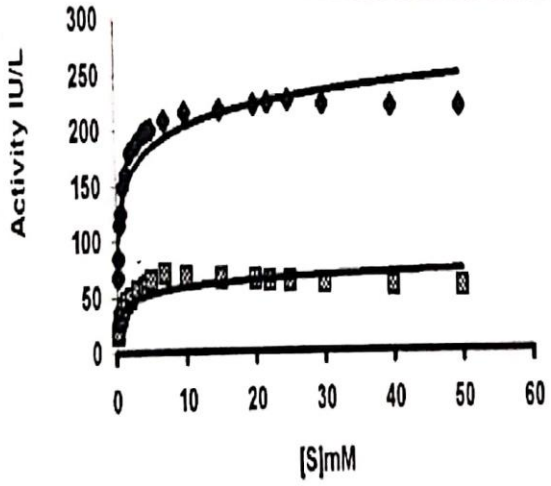

Fig. (2) Michaeles-Mentin plot for both Normal & Tuberculosis serum

Fig. (4) Hill plot for APL in both Normal & Tuberculosis serum

Fig. (3) The pseude first-order plot for S-APL enzyme in both Normal & Tuberculosis serum

Fig. (5) Arrhenius plot for S-APL in both Normal & Tuberculosis serum

مجلة ابن الهيثم للعلوم الصرفة والتطبيقية المجلد 18 (3) 2005

دراسة حركية لمعدل تفاعل الفوسفاتيز القاعدى فى التدرن الرئوى

حامد غفورى حسن و بروين عبد الصمد أسماعيل
قسم الكيمياء، كلية العلوم، جامعة السليمانية
قسم الكيمياء، كلية التربية، جامعة صلاح الدين

الخلاصة

تم بحث قيم نشاط انزيم الفوسفاتيز القاعدى فى ثلاث حالات من التدرن الرئوى هى :
١ . P , AM , EP
لقد وجد ان مستويات هذا الانزيم كانت مضطربة فى الحالات المدروسة مقارنة مع النماذج الطبيعية وذلك تحت شروط االاس الهايدروجينى القاعدى و درجة حرارة 37مئوية.
لقد ركزت الدراسة على متابعة ميكانيكية التفاعل الانزيمى حيث شملت دراسة قيم معدلات ثابت التكوين له (K1)
و مدة نصف العمر (t1/2) . لقد توصل الباحثان الى أستنتاج نمط و رتبة التفاعل الانزيمى حيث أتضح انه يخضع للعلاقة الخطية مما يدل على كونه من المرتبة الاولى. كذلك شملت الدراسة أستخراج مقدار طاقة التنشيط للمعقد الوسطى المتكون (ES) فى النماذج المرضية و الطبيعية المدروسة.