Study the effects of 3-Deoxy-3-*C*-ethoxycarbonylmethylene-1,2:5,6-di-*O*-isopropylidene-α-*D*-ribohexofuranose on normal serum alkaline phosphatase (ALP)

Firas Sh. Abdul Razzak
Chemistry Department , College of Education , Tikrit University , Tikrit , Iraq
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Abstract
The effect of 3-Deoxy-3-*C*-ethoxycarbonylethylene-1,2:5,6-di-*O*-isopropylidene-α-*D*-ribohexofuranose was tested on the activity of normal serum alkaline phosphatase (sALP) by using Different concentrations (10^{-6} - 10^{-2} M) of the compound above, the result showed different percentage of inhibitions directly proportional with the concentration and study showed Michaelis-Menten plot between [S]and [V] and Lineweaver-Burk plot between 1/[S] and 1/[V] showed (non competitive inhibition).

Key words : Alkaline phosphatase, ALP, 3-Deoxy-3-*C*-ethoxycarbonylethylene-1,2:5,6-di-*O*-isopropylidene-α-*D*-ribohexofuranose, non competitive Inhibition

Introduction
Alkaline phosphatase (EC 3.1.3.1;Ortho phosphoric-monoester phosphohydrolase, alkaline optimum;ALP) [1] is an enzyme that encompasses a family of phosphatases that carry out the enzymetic activity in alkaline environment. [2]

ALP is present in most tissues, specially liver, bones and lesser amount in intestines, placenta, kidney and leukocytes.[٣]

Measurement of serum alkaline phosphatase is useful in differentiating hepato-biliary disease from osteogenic bone disease. ALP activity increases greatly (10 times) as a result of membrane-localized enzyme synthesis after extrahepato biliary obstruction such as cholelithiasis or gallstones. Intrahepatic biliary obstruction is also accompanied by an increase ALP activity but the degree of increases is smaller. Bone disease rapid growth during puberty also elevates ALP activity as does the release of the enzyme from the placenta during the third trimester of pregnancy.[٤]

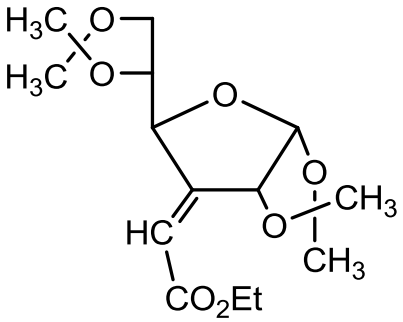
ALP requires metal ions: Mg^{+2}, Zn^{+2} and to a less extent Mn^{+2}, Co^{+2} for stability and maximum catalytic activity [٥,٦], phosphate, borate, oxalate and cyanide ions are inhibitors of all forms of the enzyme.[1]

Hydrolysis of phosphoesters, phosphate transfer activity, protein phosphatase activity, phosphate transport, modulation of organic cation transport, and involvement in cell proliferation has been suggested as possible function of ALP.[7,8]

The activity of many enzymes can be inhibited by the binding of specific small molecules. This means that inhibition of inhibiting enzyme activity serves as a major control mechanism in biological system. In addition, many drugs and toxic agents act by inhibiting enzymes.

Inhibition by particular chemicals can be a source of insight into the mechanism of system action.[٩]

Materials and Methods
3-Deoxy-3-*C*-ethoxycarbonylethylene-1,2:5,6-di-*O*-isopropylidene-α-*D*-ribohexofuranose (1) was prepared according to **Ohrui** et al.[10]



(1)

Determination of ALP activity was performed by the assay procedure according to ***DiaMond*** Diagnostic, Inc kit.

Preparation of 3-Deoxy-3-*C*-ethoxycarbonylethylene-1,2:5,6-di-*O*-isopropylidene-α-*D*-ribohexofuranose stock solution

The amount of 3-Deoxy-3-*C*-ethoxycarbonylethylene-1,2:5,6-di-*O*-isopropylidene-α-*D*-ribohexofuranose (1) used for preparation of stock solution (10^{-2} M) in (10 mL) are prepared by weighting (0.0328 gm) and dissolved in ethanol. stock solution (1 mL) was diluted in (10 mL) to give (10^{-3} M), then (10^{-4}), (10^{-5}), (10^{-6}) from (10^{-3}), (10^{-4}) and (10^{-5}) respectively. The influence of each dilution of the compound was tested on ALP activity.

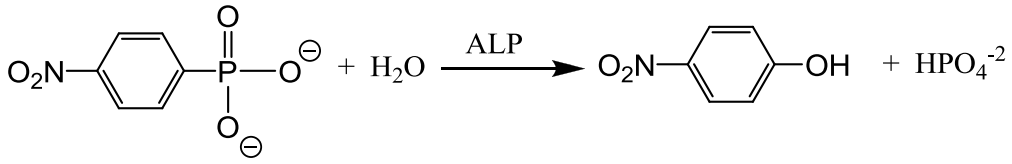
The effect of the solvent used (ethanol) for dissolving derivative (1) was tested on the activity ALP which was subtracted from the value of inhibition induced by the derivative in the solvent.

Procedure
1. The working reagent was prepared by mixing *p*-Nitrophenyl phosphate (10 mmol/L) with 50 mL of buffer Diethanolamine pH 9.8 (1 mmol/L) and Magnesium sulfate (0.6 mmol/L)
2. Aliquot of (1 mL) of reagent was pipetted into the test tubes and allowed to equilibrate to 37°C.
3. The spectrophotometer was setted to zero with absolute ethanol at 405 nm.
4. The serum sample (20 µl) was added to reagent and mixed well.
5. After one minute the absorbance was measured, the tubes were returned to the water bath at 37°C.

6. The reading were repeated every minute for the next two minutes.
7. The average absorbance difference per minute (ΔAbs/min) was calculated.

Result and discussion

Serum alkaline phosphatase , which is an important metabolic indicator, is generally quantitated by absorbance methods. ALP catalyzes the dephosphorylation of $NADP^+$ and a variety other substrates *in vivo*, the synthetic substrate *P*-nitrophenylphosphate can be used.[18]



(2) (3)

The product, nitrophenol absorbs at 405 nm with ε=1.85 × 104 M^{-1} cm^{-1}.
The Michaelis-Menten plot for inhibited and uninhibited ALP was showed in figure (1).

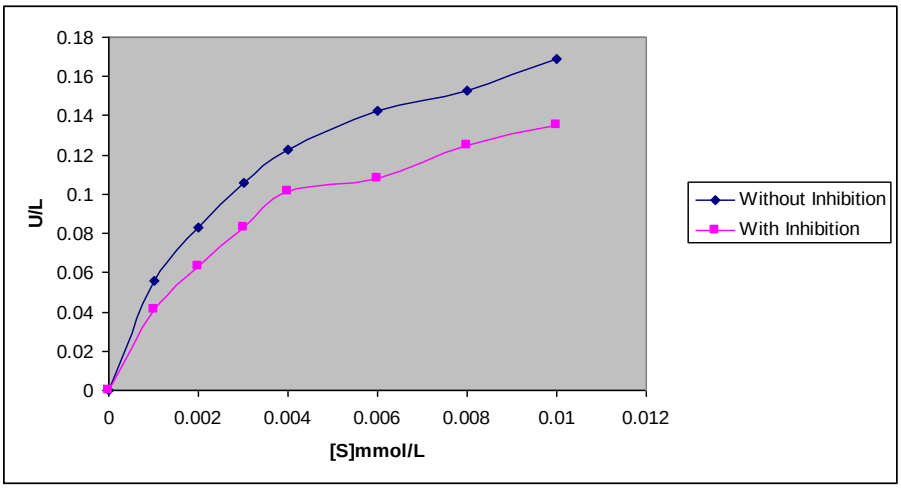


Figure (1) : Michaelis-Menten plot for inhibited and uninhibited ALP

The V_{max} value for inhibited and uninhibited ALP were (0.1353 and 0.1688 U/L) respectively, and the percentage of inhibition induced by the solvent (ethanol) on sALP was found to be 10.1 because of the denaturation of enzymes protein effecting by the solvent.
The effect derivatives(1)on sALP was shown in table (1).

Table (1) : Inhibition percentage % of derivative (1) on sALP activity

Inhibitor Concentration (M)	0.01	0.001	0.0001	0.00001	0.000001
Inhibition %	31.5	30.85	29.42	28.87	27.55

The derivative (1) showed inhibitory effect on sALP at the concentration range (10^{-6} - 10^{-2} M), the inhibitory effect was directly proportional with the concentration of the derivative under investigation, illustrated in figure (2) which shows that maximum inhibition at 10^{-2} M .

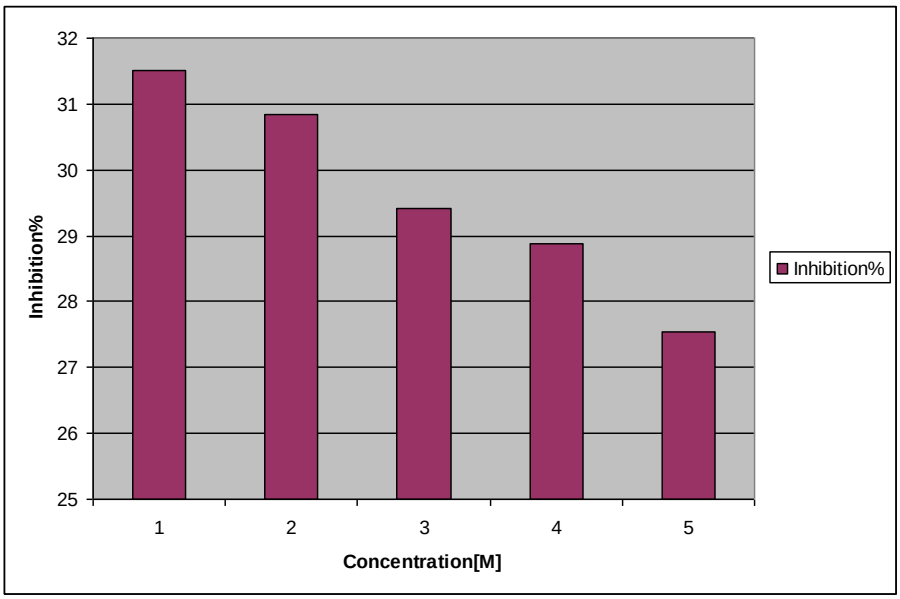


Figure (2) : Inhibition percentage for derivative (1) at diferent concentration [M] ; 1=10^{-2}, 2=10^{-3}, 3=10^{-4}, 4=10^{-5}, 5=10^{-6}

Figure (3) showed the type of enzyme inhibition using Lineweaver-Burk plot for derivative (1) on sALP activity.

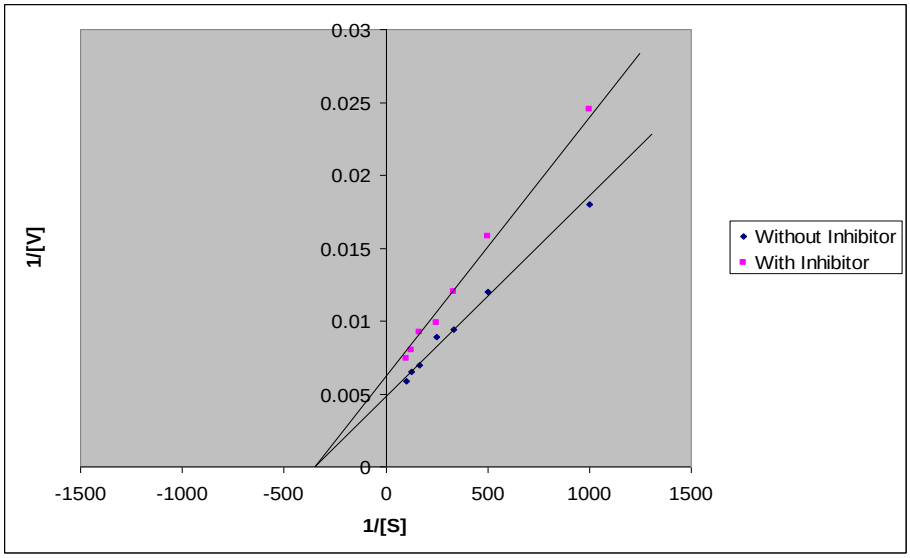


Figure (3) : Lineweaver-Burk plot for 3-deoxy-3-*C*-ethoxycarbonylethylene derivative

The derivative (1) was non competitive inhibitor for SALP activity, non competitive inhibition changes the V_{max} of the enzyme but not the K_m. The K_m value in figure (3) was 0.0028 mmol/L for the uninhibited and inhibited enzyme.

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دراسة تاثير 3-Deoxy-3-*C*-ethoxycarbonylethylene-1,2:5,6-di-*O*-isopropylidene-α-*D*-ribohexofuranose على فعالية الفوسفاتيز القاعدي في مصل دم الاصحاء

فراس شوقي عبد الرزاق

قسم الكيمياء ، كلية التربية ، جامعة تكريت ، تكريت ، العراق

(تاريخ الاستلام: ٢٧ / ٨ / ٢٠٠٨ ، تاريخ القبول: ١٥ / ١٢ / ٢٠٠٨)

الملخص:

تم دراسة تأثير المركب3-Deoxy-3-*C*-ethoxycarbonylethylene-1,2:5,6-di-*O*-isopropylidene-α-*D*-ribohexofuranose على فعالية انزيم الفوسفاتيز القاعدي(ALP) في مصل دم الاصحاء حيث استخدمت تراكيز مختلفة(10^{-2} M - 10^{-6}) من المركب المذكور حيث أظهرت نسب تثبيط مئوية تتناسب طرديا مع زيادة التركيز وتم رسم مخطط مايكلزمنتن بين التركيز وسرعة التفاعل وكذلك مخطط لاينوفر بيرك بين مقلوب التركيز ومقلوب السرعة فاظهرت تثبيطا للاتنافسي للانزيم.

الكلمات الدالة:

الفوسفاتيز القاعدي، ALP، 3-Deoxy-3-*C*-ethoxycarbonylethylene-1,2:5,6-di-*O*-isopropylidene-α-*D*-ribohexofuranose، التثبيط اللاتنافسي.