Some Aspects of Radiation Induced Damage to Enzymes Using α- amylase As A Model

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الخلاصة

تم تعريض محاليل مائية مختلفة لأنزيم ألفا اميليز لمصدر أشعة كاما للتعرف على سلوكيته تجاه الأشعة المؤينة تحت ظروف تجريبية متباينة . بوجود الأوكسجين وجد أن فعالية الأنزيم تتضاعف عدة مرات مع زيادة الجرعة الإشعاعية ، لوحظ زيادة فعالية الأنزيم عند إضافة معقدات النحاس, النيكل ، الخارصين ، وكذلك إضافة كلوريد المغنيسيوم إلى محاليل الأنزيم المائية قبل التشعيع .

هذه الظاهرة توضح بشكل جلي إحداث تغيرات في هيئة الأنزيم ، وبعبارة أخرى استحداث مناطق منفتحة تتكون نتيجة لكسر الأواصر الضعيفة التي تساهم في هيئة الأنزيم مثل الأواصر الهيدروجينية وغيرها .

تم دراسة التحلل الإشعاعي للأنزيم بوجود المعقدات الفلزية وكلوريد المغنسيوم . التحلل الإشعاعي للأنزيم في محاليل مائية مشبعة بالنتروجين لم يلحظ فيه زيادة في فعالية الأنزيم بل لوحظ انخفاض تدريجي ، وهذا يوضح دور الأوكسجين في زيادة تحسس الأنزيم تجاه الإشعاع .

درست تأثير الأيونات المعدنية على انحلال البيروكسيدات العضوية المتكونة شعاعيا في محاليل مائية مشبعة بالأوكسجين للايسين والتايروسين كنموذجين لأحماض أمينية حاوية على مخلفات اليفاتية واروماتية على التوالي .

على ضوء النتائج أعلاه أضيفت محاليل مائية مشعة للايسين بوجود معقد النحاس إلى محاليل الأنزيم فادى إلى انخفاض فعالية الأنزيم . طبقت دراسات حركية على انحلال البيروكسيدات العضوية .

خلاصة لما تقدم يمكن القول ان هذه الدراسات أعطت معلومات تبين ان فعالية أنزيم ألفا اميليز (غير المشعة) تمثل فقط جزء قليل من فعالية الأنزيم الحقيقية التي يختزنها تركيب الأنزيم وهذا

يوضح أن معظم المواقع التي تشارك في الفعالية التحفيزية للانزيم محجوبة وغير مفعلة على الأقل بصورة مباشرة .

ABSTRACT

α-amylase enzyme was isolated and purified from wheat flour (abena zero). Different aqueous enzyme solutions were exposed to gamma radiation in order to investigate the behavior of this enzyme toward ionization radiation under various experimental conditions.

Radiolysis of aqueous oxygenated α-amylase solutions led to a significant increase in activity to many folds as a function of dose.

Addition of Cu(□),Ni(□),Zn(□) complexes and $MgCl_2$ to α-amylase un-irradiated solutions also increase enzyme activity. This phenomenon indicates clearly a conformation change in enzyme structure.

Or on other word, partial unfold regions in enzyme molecule, could be created by radiation attack or/and by interactions of metal complexes or $MgCl_2$ with enzyme molecules, as a result of disruption of weak bonds such as H-banding or hydrophobic bonds.

Radiolysis of enzyme solutions containing Cu(□),Ni(□) and Zn(□) complexes and $MgCl_2$ has been investigated. Radiolysis of N_2 –saturated enzyme solutions did not show any increase in activity, but a gradual decrease was observed on proceeding irradiation. This clears up the role of oxygen in enzyme radiosensitization.

Lysine and Tyrosine amino acids were selected as model for amino acids with aliphatic and aromatic residues respectively. The effect of metal complexes on the rate of decay of organic peroxides formed on these amino acids, by irradiation was studied.

Addition of irradiated Lysine solutions in presence of $Cu^{□}(alanine)_2$ complex to un-irradiated α -amylase solution caused enzyme deactivation .

Kinetic study was applied on the decay of organic peroxides.

In brief, these studies provide information that the activity of native enzyme contributes to only small part of the whole capacity of enzyme activity. This reflects a fact that most sites involved in catalytic activity of the enzyme are not involved, at least, directly in enzyme activity.

INTRODUCTION

Enzymes are a large protein molecules essentially made up of along chain of amino acids residues in specific configuration. The radiation chemistry of enzymes is considered an important field in radiobiology because of their

biological significance.

The radiolysis of many enzymes has been studied by many workers [1-9].

Free radical inactivation of enzymes can occur by several possible mechanisms [5]. Firstly damage can occur to an amino acid residue or residues in the active site which is either involved in substrate binding or in chemistry of the catalytic reaction.

Secondary damage can occur to residues which are essential to the structural integrity of the enzyme which if disrupted can result in loss of enzyme activity. By the use of radiolytically generated free radicals, which are specific in their reactions with amino acids, it is possible to determine residues in an enzyme which are critical to enzyme function. Selective free radicals technique was used to investigate the amino acid residues which constitute the active center [1-4].

Effect of oxygen on the radiolysis of enzyme has also observed [6-8]. It was shown [6] that in vitro studies peroxy free radicals are considered to be responsible for inactivation of alcohol dehydrogenase and the cysteine and methionine residus are considered the most likely sites of initial damage. More over, an enhancement in activity was observed especially, at the early stage of irradiation in presence of oxygen [7-8].

In the present work α-amylase enzyme is recognized as important enzyme in both plant and animal kingdom [10-11]. α –Amylase enzyme affecting the quality of wheat and play an important role in the early periods of germination and splitting of starch granules in the seed .

The enzymatic actian provide maltose for yeast fermentation , and induces searecal changes in dough characteristics , [10-11]. There fore, the study of the effect of ionizing radiations on it is considered one of the most important studies in the field of enzyme radiation chemistry.

EXPERIMENTAL

Materials

Commercial Iraqi flour was obtained from one of Mosul-Flour factory and used as a source of α -amylase (saber zero).

All materials used during irradiation were of analytical grade including materials used to determine the dose rate, the preparation of metal complexes used in this research in addition to the magnesium chloride and were supplied from sigma chemical ltd.

Other compounds such as ammonium molybdate, potassium iodide and potassium hydrogen phthalate were used for peroxide determination .

Extraction and purification of α-amylase:

Extraction of enzyme has been carried out using method described in Ref [12], Optimum pH and temperature were also obtained from Ref[13].

Optical pH and Optical Temperature :

Optical pH of α - amylase from Iraqi wheat flour (sabir zero) was determined using soluble starch and acetate buffer of pH 3.5 – 7.5 Reaction time used was (15 min) at optimum temp . The optical temperature was determined at optical pH ranging between (10 – 100 ^{0}C) for temperature and (3.6 – 6.8) pH using acetate buffer .

Determination of α -amylase activity:

α -amylase activity was determined using the dinitrosalicylic acid method of Bendlows [18] at its optimum pH (pH 6) and optimum temperature (20°C).

Maltose was determined by Nelson's colorimetric copper method using a malyose standard cure soluble starch was used as sustsate .

Preparation of Metal complexes:

The following complexes were prepared according to the literature as indicated below :

-Bis-(L-alanino) Copper (Π)[Cu^{Π}(alanine)$_2$].

2-Bis-(L-alanino) nickel (Π) tri hydrate [Ni^{Π}(alanine)$_2$] $3H_2O$ [14].

3-Bis-(L-histidine) nickel (Π) monohydrate [Ni^{Π}(histidine)$_2$] H_2O [15].

4-Bis-(L-alanino) Zinc (Π) [Zn^{Π}(alanine)$_2$] [14].

Preparation and irradiations of solutions:

All solutions were prepared using freshly triple-distilled water.The ordenary distillation was followed by another distillation from alkaline potassium permanganate solution, then redistill ordinary once again to obtain triply-distilled water with conductivity not more than $3x10^{-6}$ ohm^{-1} cm^2).

The freshly prepared oxygenated or N_2-saturated enzyme solutions, and oxygenated Lysine and Tyrosine aqueous solutions were irradiated at room temperature (25°C) for thirty minutes .

All γ-irradiations were carried out using gamma cell-220, purchased from Canadian atomic Energy. The source consists of a number of 60 Co rods.

All spectrophotometer measurements were carried out using Shimadzu UV- visible Recording spectrophotometer. UV 160.

The pH measurements were performed on Philips PW 9420 meter.

The dose rate used was $4.5x10^{16}$ eV ml^{-1} min^{-1}.

Determination of peroxides:

Total peroxides (TP) include organic peroxide (OP) and H_2O_2. The most widely used method for determination of peroxides (TP) in aqueous solutions is the iodide method which is based on that of Allen et al [16] .It involves the oxidation of potassium iodide by H_2O_2 and organic peroxidic materials in presence of ammonium molybdate as catalyst.

The liberated iodine is determined spectrophotometrically as I_3^- at 352nm $\varepsilon = 2.4x10^3$ dm^3 $mol^{-1}cm^{-1}$.

H_2O_2 is determined directly by Tinder method (peroxidase method) [17]. The method which based on the reaction of H_2O_2 with 4-amino antipyrine-phenol in presence of peroxidase as catalyst.

RESULTS AND DISCUSSION

Gamma radiolysis of α-amylase aqueous solutions under various experimental conditions has provided some new interest results about the behavior of protein upon the effects of ionizing radiation. Many experiments were performed to give enough evidence to draw a satisfactory mechanism pathway for the effects of ionizing radiation on the enzyme.

A-Effect of γ -irradiation on α -amylase aqueous oxygenated solutions:

Samples of α-amylase aqueous oxygenated solutions were irradiated at pH 6 and 25°C using different irradiation doses. The obtained results were summarized in Table (1) the observed activity values of the enzyme immediately after each certain dose were plotted as function of dose. The decrease in enzyme activity at the early stage of irradiation is occurred due to reactions of water primary active species (in oxygenated solutions oxygen scavenge both $H^\cdot$ and e_{aq}^- leaving OH radicals) with amino acids residues in the enzyme which are crucial to activity, by increasing time of irradiation, the enzyme structure can be broken up [5] to expose amino acids residues involved in substrate binding or in the chemistry of catalytic reaction of enzyme activity, led to the observed increase in activity as shown in Table (1), since a large increase in activity has been observed it was about nine times of that activity for the enzyme before irradiation (native form) .Once again , the change in structure integrity of the enzyme during irradiation in presence of oxygen was companied with the formation of peroxides (Table 2) which can be formed according to the following reaction steps.

gamma

$H_2O \xrightarrow{\text{radiation}} (H_2O^+, e^-, H_2O^*) \longrightarrow {}^{\cdot}OH, e_{aq}^-, H^{\cdot}, H_2, H_3O^+, H_2O_2$ .... (1)

In presence of oxygen, oxygen scavenge both solvated electrons and hydrogen atoms [19].

$$e_{aq}^- + O_2 \longrightarrow O_2^{\cdot -} \quad \text{..... (2)}$$

$$H^{\cdot} + O_2 \longrightarrow HO_2^{\cdot} \quad \text{..... (3)}$$

$$HO_2^{\cdot} \rightleftharpoons H^+ + O_2^{\cdot -} \quad \text{..... (4)}$$

Thus, hydroxyl radicals attack enzyme including addition or abstraction reactions as follows:

$$^{\cdot}OH + E \longrightarrow E(OH)^{\cdot} \xrightarrow{O2} E(OH)O_2^{\cdot} \quad \text{.....(5)}$$

$$^{\cdot}OH + E \xrightarrow{-H2O} E(-H)^{\cdot} \xrightarrow{O2} E(-H)O_2^{\cdot} \quad \text{.....(6)}$$

were E represents the enzyme molecule .

The so formed radicals can be converted to peroxide by reduction processes:

$$E(OH)O_2^{\cdot} + O_2^{\cdot -}/HO_2^{\cdot} \longrightarrow E(OH)O_2H + O_2 \quad \text{.....(7)}$$

$$E(-H)O_2 + O_2^{\cdot}/HO_2^{\cdot} \longrightarrow E(-H)O_2H + O_2 \quad \text{.....(8)}$$

Thus, irradiation in oxygenated conditions offer clear evidence for the significant effect of oxygen as a good radio sensitizer, in addition, oxygen is precursor for peroxides formation. Which upon its decay leads for further modifications involving both damages of enzyme active sites as well as a conformational change on enzyme structure that leads to significant changes in activity.

Table 1

Effect of γ - irradiation on the activity of α-amylase in aqueous oxygenated solutions using dose rate of 4.5 10^{16} ev/ ml / min^{-1} .

Time (minute)	Activity (mu / ml)
0	1606
5	1510
10	1253
15	1207
20	2336
25	3410
30	4088
35	8275
37	10275
40	13.549
42	9214
45	7128
50	2470
55	2025
60	1621

Table 2

Determination of peroxides formed by the γ - irradiation of Oxygenated aqueous solutions of α - amylase at pH 6 and 25C°.

Dose X 10^{-16} ev / ml / min	G (TP)*
4.5	2.8
9.0	2.6
13.5	2.5
18.0	2.5
27	2.2

The results listed in this table are qualitative rather than quantitative because of the difficulty of the exact determination of peroxides on enzyme

B-Effect of some metal Complexes on the activity of α-amylase before, during and after irradiations in aqueous oxygenated solutions:

For further information, transition metal complexes such as $[Cu^{II}(alanine)_2]$,$[Ni^{II}(alanine)_2]3H_2O$, $[Ni^{II}$ $(histidine)_2]$ H_2O and Zn^{II} $(alanine)_2$ were prepared and added separately to enzyme aqueous

oxygenated solutions to investigate their effects before , during and after gamma irradiations .

Table (3) shows clearly that these complexes caused a large increase in enzyme activity before irradiation. These observations can be explained in the view of the enzyme structure integrity, since the interactions of these complexes seemed to induce a certain effect that cause to open the structure of enzyme which can lead to expose more amino acids residues which involved in the activity of enzyme. By the same time, this condition make the free radical enzyme inactivation easier by reactions of hydroxyl radicals with amino acid residues containing the active sites [5] .

The results supported this suggested mechanism pathway since a great decrease in activity was noticed as a function of irradiation doses. Table **(4)** shows the effects of Cu^{II}(alanine) and $Ni^{II}(alanine)_2\ 3H_2O$ on the activity of α-amylase during irradiations . In the same experiments, monitoring the activity after irradiation as a function of time (in presence of Cu^{II} $(alanine)_2$ complex) showed a further decrease in activity followed by an increase as shown in Table **(5)** . The observed decrease in activity as post-irradiation effects can be occurred through peroxides decomposition induced free radicals formation that cause further damage on enzyme. Once again, the observed increase in activity, as explained above due to further structural changes a long the enzyme molecules. On the light of these results and on the light of already observed process [20], including reduction of complexes by solvated electrons followed by replacement of ligand by water molecules, a following mechanism pathway can be suggested:

$$O_2^{\cdot-} + M^{II}(L)_2 \longrightarrow M^{I}(L)_2 + O_2 \quad .....(9)$$

The latest reaction (reaction 9) indicates also the superoxide dismutation reaction that can be occurred by complexes used in this work [21].

However, a fast replacement of ligand with water molecules can be occurred as follows:

$$M^{I}(L_2 + 2H_2O \longrightarrow M^{I}(L(H_2O)_2) + L \quad .....(10)$$

$$M^{I}(L(H_2O)_2) + 2H_2O \longrightarrow M^{I}(H_2O)_4 + L \quad .....(11)$$

$$M^{I}(H_2O)_4 \longrightarrow M^{I}_{aq} \quad .....(12)$$

The reduced metal ions (i.e ,Cu^{I},Ni^{I} ,Zn^{I}) can undergo fast oxidation by reactions with enzyme –OH radical adducts resulting in regenerate the more stable divalent metal ions and by the same time a coordination with the

enzyme can be taken place through the oxidation –reduction processes . Which eventually lead to streriochemical changes on enzyme molecule causing to increase activity.

This phenomenon is well illustrated by comparing these results with those obtained from irradiation enzyme free oxygen solutions (see Table 15) and are discussed below .

Table 3

Effects of Cu(Π) , Ni(Π) , and Zn(Π) complexes ($3X10^{-4}$M) on the activity of un-irradiated α- amylase

Complex	Activity (mu/ml)
Non	1602
Cu^{Π} $(alanine)_2$	5732
Ni^{Π} $(alanine)_2$ $3H_2O$	5720
Ni^{Π} $(histidine)_2$ H_2O	5718
Zn^{Π} $(alanine)_2$	5724

Table 4

Effect of $Cu^{\Pi}(alanine)_2$ and $Ni^{\Pi}(alanine)_2$ 3H 2O ($3X10^{-4}$ M) Complexes on the activity of α- amylase during irradiation . Using dose rate of 4.5×10^{16} ev/min /ml .

Irradiation time (minutes)	Activity of α- amylase (mu/ml)	
	Cu^{Π} $(alanine)_2$	Ni^{Π} $(alanine)_2$ $3H_2O$
1	4995	4842
3	4721	4961
5	4433	4321
7	4120	3975
10	3872	3742
15	3632	3520
20	3481	3384
25	3148	3001
30	2719	2682

Table 5
Post – irradiation effect of Cu^{II}(alanine $)_2$ complex on the activity of α - amylase in aqueous oxygenated solutions using dose of 1.35 X 10^{18} ev1/ml .

Time (minutes)	Activity of α - amylase
0	2719
5	2921
10	2870
15	2816
20	2542
25	2127
30	1705
35	1942
40	2094
45	2223
50	2512
55	3241
60	4245

C- Effects of the complexes on irradiated α-amylase solutions:

Addition of metal complexes (mentioned above $3x10^{-4}M$) to irradiated enzyme solutions, using dose of $1.35x10^{18}$ ev m^{l-1} affected the enzyme activity. It is clear from the results that all metal ion complexes caused an increase in activity followed by gradual decreases and the effects of these complexes were in the following orders:

$$Ni^{II}(histid)_2 3\,H_2O > Ni^{II}(alanine)_2\,3H_2O > Cu^{II}\,(alanine)_2 >> Zn^{II}\,(alanine)_2$$

The gradual decrease in enzyme activity in irradiated aqueous solutions in presence of metal complexes can be explained on the bases of peroxide decomposition.

Since metal complexes expected to increase the decay of organic peroxides that formed on enzyme (Table 6) during gamma irradiation in oxygenated aqueous solutions. To make sure about this suggestion, experiments were performed by studying the effect of these complexes on the decay of organic peroxides formed in gamma radiolysis of some amino acids.

Table 6
Post – irradiation effects of Cu(II), Ni(II), and Zn(II) complexes ($3X10^{-}$ M) on the activity of α- amylase using dose of $1.35X10^{18}$ ev/ml.

Time (minutes) after irradiation	Activity of α- amylase mu/ml			
	$Cu^{II}(alanine)_2$	$Ni^{II}(alanine)_2 3H_2O$	$Ni^{II}(histid)_2H_2O$	$Zn^{II}(alanine)$
0	5730	5720	5724	5718
15	5256	5202	5121	5231
30	4902	4584	4364	4827
45	2968	2878	2452	4427
60	2298	2133	1827	4027

D-Effects of the above metal complexes on the organic peroxides produced on gamma radiolysis of Lysine and Tyrosine in aqueous solutions:

Enzyme consists of many different amino acids residues, primary water radicals will react at many different sites in enzyme because the general lack of specificity of reaction of these radicals with amino acids moieties. Thus, Lysine used as a model for aliphatic amino acids and Tyrosine was used as model for amino acids with unsaturated residues. Thus, the yield of peroxides formed on these selected amino acids (Table 7) is well agree with that has been already reported [22] can be in general represented all types of peroxides in the enzyme. Tables (8 & 9) , obviously indicated the effect of the metal complexes on the decay of organic peroxides on Lysine and Tyrosine respectively under conditions similar to that in the case of α-amylase . It was found that the decay of organic peroxides is increased as a function of time and also by increasing metal complexes concentrations. From these experiments, it can be concluded that the effect of metal complexes on activity of enzyme after irradiation is due to the decomposition effect of organic peroxides on enzyme and by the same time, the results indicated a formation of new free radical specially ·OH radicals. The following mechanism can be suggested to explain the post-irradiation effect of metal complexes on peroxide decay.

$$ROOH + M(L_2)^{2+} \longrightarrow M^{1+} + ROOH^{\cdot}$$

$$ROOH^{\cdot} \xrightarrow[-OH^{-}]{H_2O} ROH + {}^{\cdot}OH$$

The expected formation hydroxyl radicals explained the observed further modification in enzyme and by the same time, the observed further change in enzyme activity also gave evidence for the formation of ·OH radicals in

these systems. The effect of metal ion complexes was found to vary depending on both, the type of metal ion and the ligand used.

Table 7
Determination of peroxides (TP and OP) produced in radiolysis of Tyrosine and Lysine amino acids ($2X10^{-3}$ M) in aqueous oxygenated solutions at pH 6.

Amino acid	G(TP)	G(OP)	$G(H_2O_2)$
Tyrosine	2.1	1.2	0.9
Lysine	2.08	1.22	0.86

Here G represents the number of peroxide molecules produced per 100ev of absorbed dose.

Table 8
Effects of Cu(II) and Ni(II) complexes on the decay of organic peroxides produced in γ- radiolysis of Tyrosine oxygenated solutions at pH 6 and 25C°.

Time (minute) after irradiation	% of Decay of organic peroxides					
	$Cu^{II}(alanine)_2$		$Ni^{II}(alanine)_2 3H_2O$		$Ni^{II}(histidine)_2 H_2O$	
	$3X10^{-5}M$	$3X10^{-4}M$	$3X10^{-5}M$	$3X10^{-4}M$	$3X10^{-5}$ M	$3X10^{-4}$ M
10	7.6	10.3	8.2	11.2	10.1	13.1
20	9.3	17.1	10.3	17.4	12.4	18.8
30	16.4	31.4	17.2	33.8	19.4	34.7
40	30.8	40.8	32.3	41.9	32.8	46.5
50	33.6	52.9	36.7	56.2	39.4	60.2
60	40.1	60.3	46.2	67.3	50.1	62.3

Table 9
Effects of Cu(II),and Ni(II) complexes on the decay of organic peroxides produced in γ-radiolysis of Lysine oxygenated aqueous solutions at pH 6 and 25C°.

Time (minute) after irradiation	% of Decay of organic peroxides					
	Cu^{II} (alanine)$_2$		$Ni^{II}(alanine)_2 3H_2O$		$Ni^{II}(histidine)_2 H_2O$	
	$3X10^{-5}M$	$3X10^{-4}M$	$3X10^{-5}M$	$3X10^{-4}M$	$3X10^{-5}M$	$3X10^{-4}M$
10	9.1	16.2	10.3	17.2	11.3	18.4
20	11.4	19.7	13.2	19.9	14.3	22.4
30	15.2	37.2	17.7	36.4	17.8	41.0
40	21.1	56.4	23.4	52.6	25.4	56.7
50	29.6	62.4	32.4	59.4	37.5	62.8
60	46.7	69.2	49.2	71.2	35.1	67.4

Application of kinetic laws on the results derived from the study of metal complexes on some amino acids organic peroxides (Lysine and Tyrosine organic peroxides) show that at relatively low metal complexes concentration ($3x10^{-5}$ M) , the decay of organic peroxides follow mainly second order kinetic at room temperature (25°C) . At relatively high complex concentration ($3x10^{-4}$ M), mainly pseudo-first order kinetics plots were obtained (Figures 1-10) . The results of rate constants listed in Table (10,11) show that $Cu^{II}(alanine)_2$ H_2O is the most effective complex upon the used metal complexes in this research . As mentioned above ,·OH radicals are presumably formed during organic peroxide decomposition . To prove this suggestion and by the same time, to illustrate the effect of the expected formed radicals on enzyme activity the effect of Lysine organic peroxides on enzyme activity was studied in presence of $Cu^{II}(alanine)_2$ complex

Table 10

Rate constants (k= sec^{-1}) of decay of organic peroxides formed in γ- irradiation of oxygenated aqueous solutions of Tyrosine and Lysine ($2x10^{-2}$M) at pH 6 and 25 C° in presence of Cu(II) and Ni(II) complexes at different concentrations.

Amino acids	Rate constants k : sec^{-1}					
	$Cu^{II}(alanine)_2$		$Ni^{II}(alanine)_2 3H_2O$		$Ni^{II}(histidine)_2 H_2O$	
	$3X10^{-5}$ M	$3X10^{-4}$ M	$3X10^{-5}$ M	$3X10^{-4}$ M	$3X10^{-5}$ M	$3X10^{-4}$ M
Tyrosine	$1.375x10^{-4}$	$2.5x10^{-4}$	-	$2.375x10^{-4}$	-	$2.75x10^{-4}$
Lysine	-	$3.25x10^{-4}$	-	$3.17x10^{-4}$	$1.167x10^{-4}$	$3.33x10^{-4}$

Table 11

Rate constant (k: dm^3 mol^{-1} sec^{-1}) of decay of organic peroxides formed in the irradiation of oxygenated aqueous solutions of Tyrosine and Lysine ($2X10^{-3}$M) at pH 6 and 25° C in presence of Cu (II) and Ni(II) complexes at different concentrations .

Amino acids	Rate constants k:dm^3 mol^{-1} sec^{-1}					
	$Cu^{II}(alanine)_2$		$Ni^{II}(alanine)_2 3H_2O$		$Ni^{II}(histidine)_2 H_2O$	
	$3X10^{-5}$ M	$3X10^{-4}$ M	$3X10^{-5}$ M	$3X10^{-4}$ M	$3X10^{-5}$ M	$3X10^{-4}$ M
Tyrosine	7.33	15.15	7.78	10.7	10.1	20
Lysine	8.13	-	9.39	-	5.13	-

E- Effect of Lysine organic peroxides on enzyme activity in presence of $Cu^{II}(alanine)_2$ complex:

For further evidences about the mechanism path way of peroxides decomposition effect on enzyme activity, an experiment was done in which organic peroxides were prepared radiolytically by radiolysis of Lysine oxygenated solution and then was added to un-irradiated enzyme solution in presence of $Cu^{II}(alanine)_2$ complex (10^{-4} M) . The results in Table (12) show that a decrease in activity was observed as a function of time , consequently as function of peroxide decomposition . These results can be compared with that in Table (9) which indicated clearly the effect of peroxide formation on the activity of enzyme .

Table 12

Effect of lysine organic peroxides (produced in γ – irradiation of aqueous oxygenated Lysine solutions on the activity of un-irradiated α- amylase in presence of $Cu^{II}(alanine)_2 3H_2O$ complex ($3x10^{-4}$M) .

Time (minutes) after addition of peroxides	Activity of α- amylase
0	5620
5	5224
10	4922
15	4632
20	4343
25	4122
30	3918
35	3722
40	3622
45	3372
50	3234
60	3028

Application of kinetic laws on the observed enzyme decomposition considering the initial enzyme activity as initial concentration was performed and first order plot is obtained (Figure 11) , (Table 13).

The following arguments can be drawn to fit the kinetic results:

1-Formation of oxidizing radicals through organic peroxide decay induced by metal complexes.

2- About two- third of presumably formed ·OH radicals caused deactivation which means that at least two –third of ·OH radical targets constitute the active sites of the enzyme.

3- On the light of results obtained, it can be said here, that most of active sites are not involved in the activity of enzyme since they are shielded by structure of enzyme.

4- The apparent enzyme activity (Activity observed in absence of any additions and before irradiation) is indeed contributes to only small part of whole capacity of real enzyme activity.

CONCLUSION

On the light of the present results , important information was extracted. From the fact of view that polypeptide chain containing many residues could in principle, fold into many conformations. In general, however, all molecules of any protein species adopt a single conformation called native state, which is more stable fold form of the molecule. The native state is maintained through non-covalent bonds between the amino acids and any prosthetic groups. Protein unfolds or denature under various condition that disrupt the weak bonds [23]. Thus, reaction of water active species,·OH ,e_{aq}^- ,H . in absence of oxygen and only OH radicals in presence of oxygen (see discussion part) with amino acids residues cause a different changes in activity of α-amylase enzyme as already shown .It is obviously shown that a change in conformation must be taken place upon irradiation in presence of oxygen or metal complexes and that change sensitize the enzyme causing a considerable increase in activity . However, these observations are indeed including partial unfolded. Complete unfolded leads to enzyme inactivation as in the case of anoxic condition (N_2-saturated).

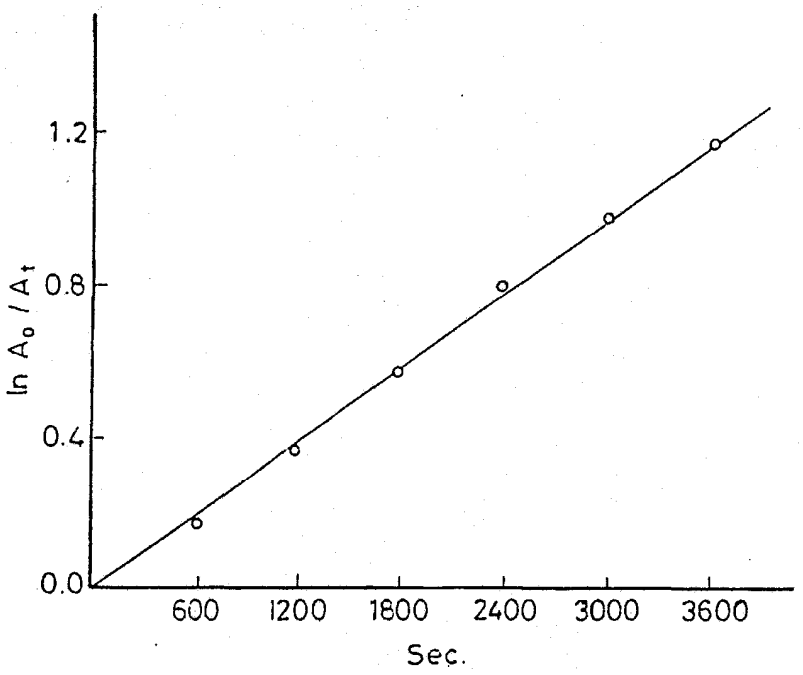
Decomposition of organic peroxide materials in presence of metal ions or metal complexes leaves a free radicals on carbon center [24] . Thus, the decomposition of organic peroxide materials which was found in enzyme molecules particularly those produced on sulfur a tom (-RSO_2H) can lead to form –RS· radicals that eventually reform the disulfide bridge in enzyme [25]. The new form disulfide bonds may have positions differ from original before irradiation, which reflect new behaviors.

The assumption of formation of partial unfolded enzyme is expected to form due to complex formation, which is already discussed. This may cause a geometrical inversion in enzyme structure, similar to that expected to occur in

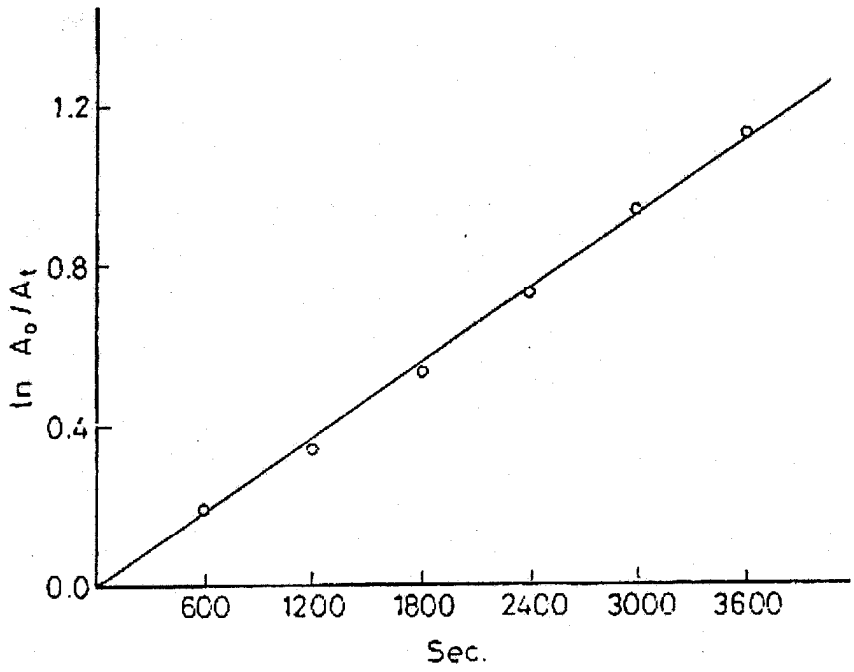
DNA [26] .In the case of irradiation in presence of oxygen , the nature of folded protein adds possibilities of fainting of peroxy radicals in addition to that mentioned before in this research .

The following possibilities may fit the obtained data under various experimental conditions .An intra H-atom abstraction from aliphatic part or electron transfer from convenient parts of amino acids in a well geometrical distribution. Furthermore addition of peroxy radicals at any convenient double bond in amino acids in the enzyme. These processes lead to generate new radicals, and consequently, a sort of chain reactions can be then started.

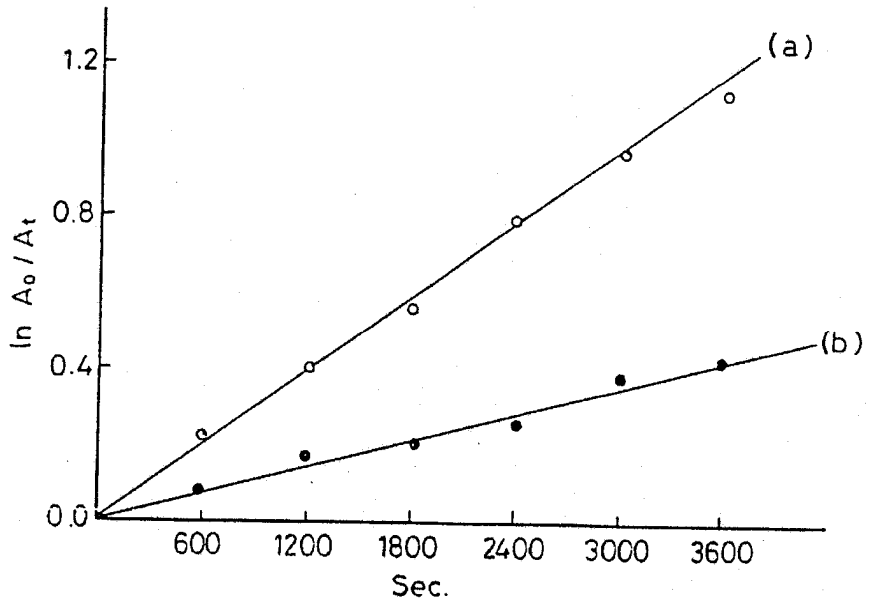
However, these interactions can lead to unfold protein and a channel could be produced a long the enzyme molecule make many amino acids residues of the enzyme active sites ready to involve in the catalytic activity that reflect a significance increase in enzyme activity. By the same time this case introduce a good condition lead to high damage on further irradiation by the easy reactions of water free radicals with more exposure amino acids residues that constitute the active



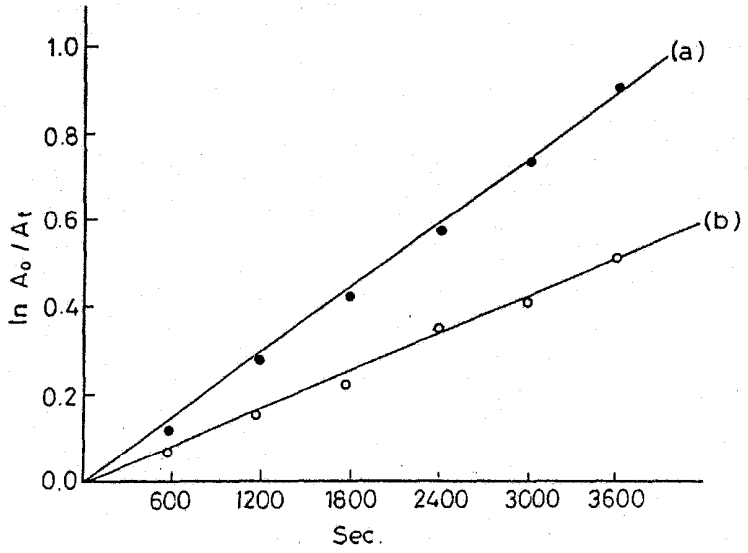
Fig(1). First-order decay of organic peroxides formed in the γ-irradiation of oxygenated aqueous solutions of Lysine (2×10^{-3}M) at pH 7 and 25 °C in presence of $Cu^{II}(alanine)_2$ complex (3×10^{-4} M)



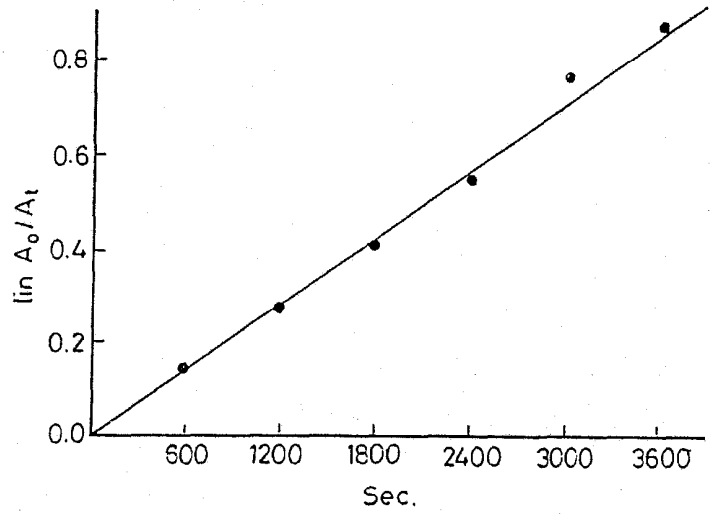
Fig(2). First-order decay of organic peroxides formed in the γ-irradiation of oxygenated aqueous solutions of Lysine (2×10^{-3}M) at pH 7 and 25 °C in presence of $Ni^{II}(alanine)_2$ complex (3×10^{-4} M)



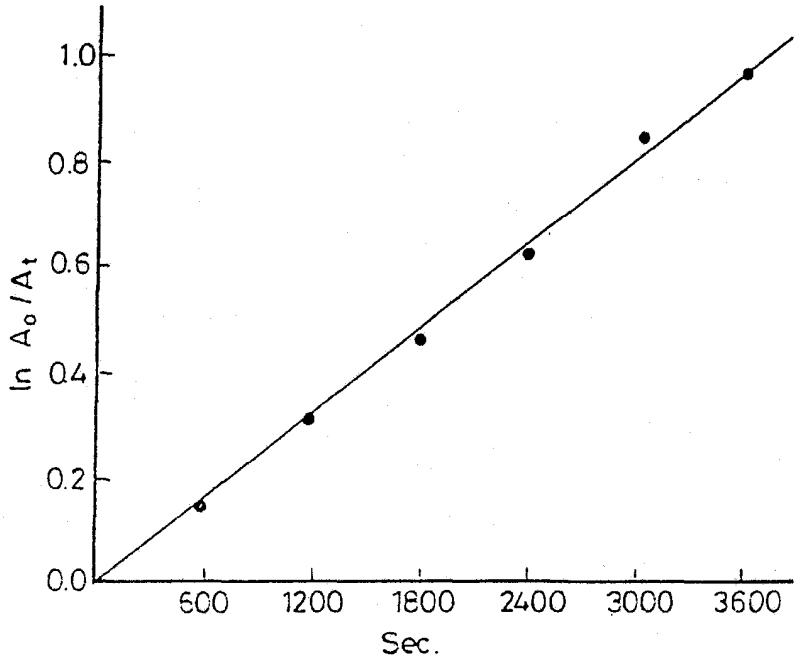
Fig(3). First-order decay of organic peroxides formed in the γ-irradiation of oxygenated aqueous solutions of Lysine (2×10^{-3}M) at pH 7 and 25 °C in presence of $Ni^{II}(histidine)_2H_2O$ complex
a: (3×10^{-4} M) b: (3×10^{-5} M)



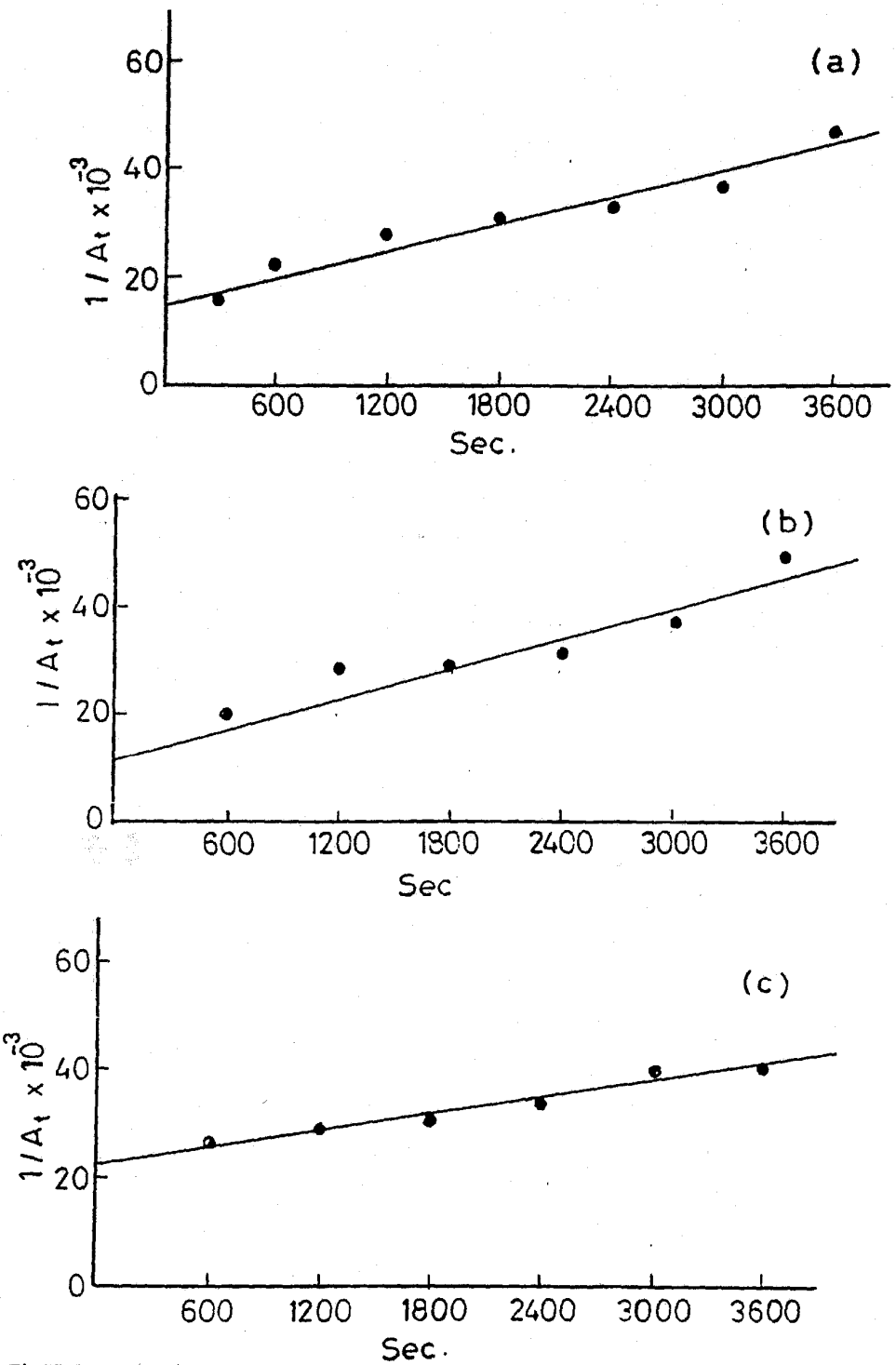
Fig(4). First-order decay of organic peroxides formed in the γ-irradiation of oxygenated aqueous solutions of Tyrosine (2×10^{-3}M) at pH 7 and 25 °C in presence of $Cu^{II}(alanine)_2$ complex
a: (3×10^{-4} M) b: (3×10^{-5} M)



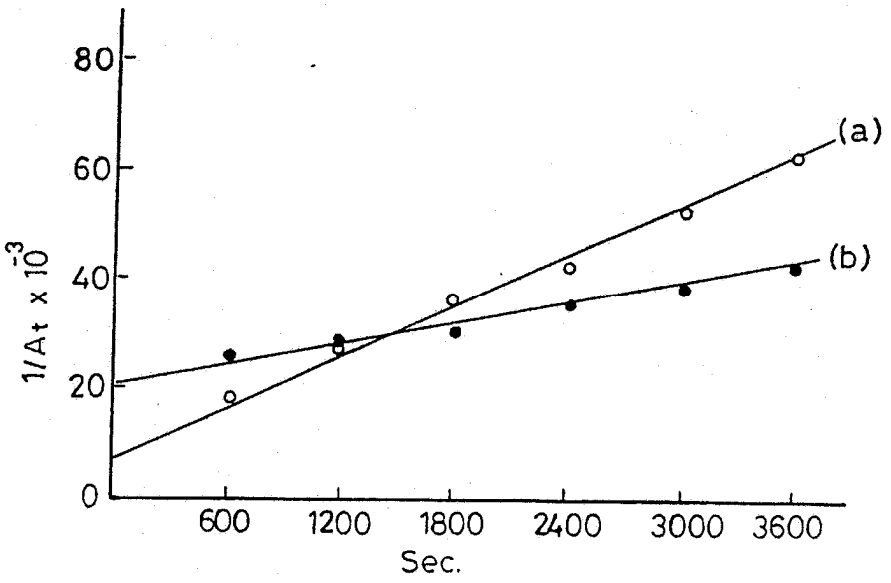
Fig(5). First-order decay of organic peroxides formed in the γ-irradiation of oxygenated aqueous solutions of Tyrosine (2×10^{-3}M) at pH 7 and 25 °C in presence of $Ni^{II}(alanine)_2 3H_2O$ complex (3×10^{-4}M)



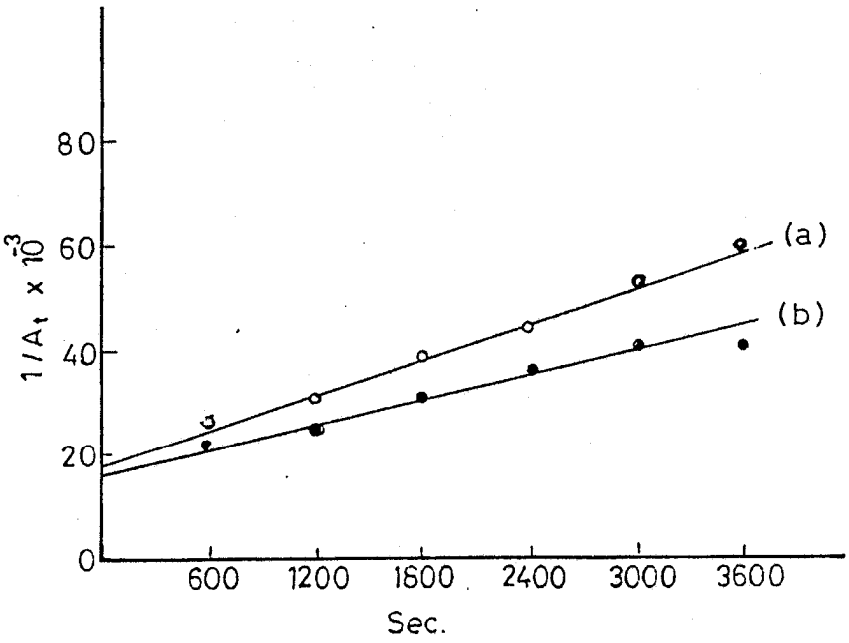
Fig(6). First-order decay of organic peroxides formed in the γ-irradiation of oxygenated aqueous solutions of Tyrosine (2×10^{-3}M) at pH 7 and 25 °C in presence of $Ni^{II}(histidine)_2H_2O$ complex (3×10^{-4}M)



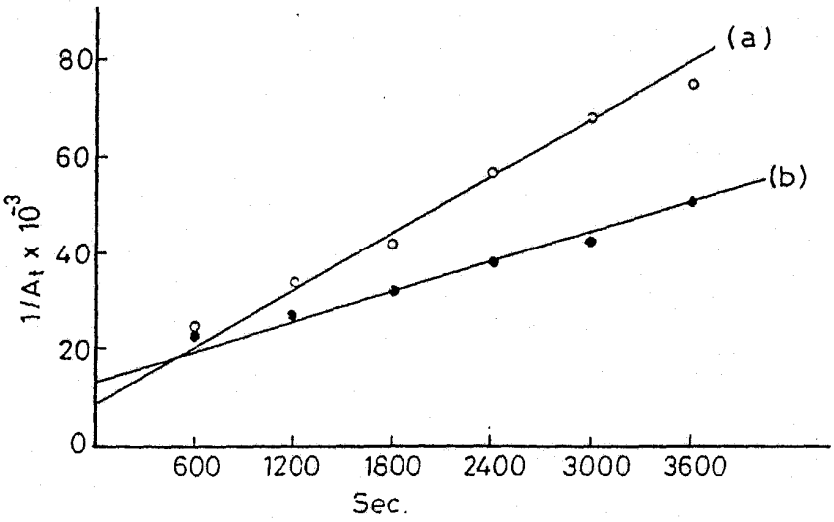
Fig(7).Second-order decay of organic peroxides formed in the γ-irradiation of oxygenated aqueous solutions of Lysine (2×10^{-3}M) at pH 7 and 25 °C in presence of metal complexes (3×10^{-5}M)
a: $Cu^{II}(alanine)_2$ b: $Ni^{II}(alanine)_2 3H_2O$ c: $Ni^{II}(histidine)_2 H_2O$



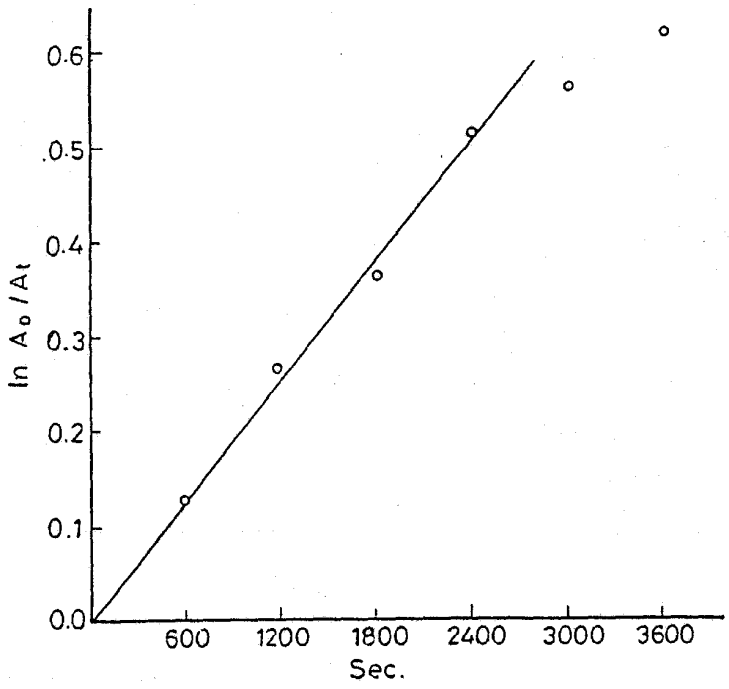
Fig(8). Second-order decay of organic peroxides formed in the γ-irradiation of oxygenated aqueous solutions of Tyrosine (2×10^{-3}M) at pH 7 and 25 °C in presence of $Cu^{II}(alanine)_2$ complex
a: (3×10^{-4} M) b: (3×10^{-5} M)



Fig(9). Second-order decay of organic peroxides formed in the γ-irradiation of oxygenated aqueous solutions of Tyrosine (2×10^{-3}M) at pH 7 and 25 °C in presence of $Ni^{II}(alanine)_2 3H_2O$ complex
a: (3×10^{-4} M) b: (3×10^{-5} M)



Fig(10). Second-order decay of organic peroxides formed in the γ-irradiation of oxygenated aqueous solutions of Tyrosine (2×10^{-3}M) at pH 7 and 25 °C in presence of $Ni^{II}(histidine)_2 3H_2O$ complex
a: (3×10^{-4} M) b: (3×10^{-5} M)



Fig(11). First-order enzyme deactivation induced by the decay of Lysine organic peroxides in presence of $Cu^{II}(histidine)_2$ complex (3×10^{-4} M).

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