J. Pest Control & Environ. Sci. Vol 2 pp 1-8, 1990
Symposium of IPM & E.P. Nov.7-8, 1990 Alex. Egypt.

Lipoxygenase Enzyme a New Target for Nicotine and Rotenone Insecticides

By

Nader Shaker[1] and Nadia Shahban[2]

[1]Pesticide Dept., Faculty of Agriculture and [2]Biochemistry Dept., Faculty of Science. University of Alexandria, Alexandria, Egypt.

Abstract

Lipoxygenase (linoleate oxygen oxidoreductase) activity has been measured in the presence and absence of nicotine and rotenone botanical insecticides.

Nicotine inhibits lipoxygenase enzyme with a time and concentration dependent response with I_{50} value equal to 5×10^{-7} M concentration. Lineweaver-Burk plot design shows that nicotine inhibits lipoxygenase enzyme with a value of K_m equal 0.66 µM in a non-competitive inhibition manner with a V_{max} equal 18.5 µM/mg protein/min in the presence of 25 µM of nicotine concentration.

Rotenone inhibits lipoxygenase enzyme in a dose dependent way after a preincubation period between the enzyme protein and rotenone with I_{50} value equal to 2×10^{-7} M concentration. Rotenone inhibits lipoxygenase enzyme with non-competitive inhibition manner with K_m value of 0.66 µM and V_{max} 30 and 20 µM/mg protein/min in absence and presence of 2.5×10^{-5} M concentration.

Specific and selective inhibition of lipoxygenase enzyme by nicotine and rotenone is useful not only as a tool for investigating regulatory mechanism of leukotriene biosynthesis but also as a new site of toxicological action of this group of insecticides.

Introduction

Lipoxygenase [E.C. 1.13.11.12] linoleate oxygen oxidoreductase in a non-heam iron dioxygenase enzyme that catalyzes the oxidation by molecular oxygen of certain polyunsaturated fatty acids that contain cis-cis 1-4 pentadiene moiety such as linoleic acid to form the conjugation of cis-trans diene hydroperoxide (Eskin et al., 1977).

Much of the interest in lipoxygenase is due to its importance in the post harvest of many food products. Lipoxygenase is also thought to be important in the formation of flavors of many fruits and vegetables, i.e., cucumber, tomatoes, melons ... etc (Dauillard and Bergesn, 1981).

This enzyme is involved in the formation of a biradical from linoleate and oxygen on the surface of the enzyme (Tappel et al., 1953). Inhibition of lipoxygenase enzyme (Baumann et al., 1980) by nicotine, rotenone, or any other inhibitor will initiate the free radical chain reaction in the biological system (Vliegenthart and Veldink, 1982). This will be associted with an increase of several deleterious effects in animals and plants including tumor growth (Borg, 1970), cancer (Fridovich, 1976 and Cohen, 1978), neural disorders and inflamation effect (Siegel et al., 1980).

The present study was undertaken to investigate lipoxygenase as a new target for the toxicity of nicotine and rotenone insecticides.

Materials and Methods

Linoleic acid (highly pure) provided by Sigma was used to prepare sodium linoleate by using 70 mg of linoleic acid with an equal weight of Tween-20, both were added to O_2-free water. The mixture was homogenized avoiding air bubbles, then measured and equivalent amount of 0.5 N NaOH was added and the total volume made up to 25 ml by distilled water. The total volume was divided into 1-2 ml portions in small screw-cap vials, flushed with nitrogen before closing and were kept frozen until used.

Nicotine, chemically pure was supplied by Merck, and rotenone as a pure sample was provided by EPA, USA.

Lipoxygenase enzyme was prepared from Soya beans according to Christopher et al. (1972) method.

Enzyme assay was carried out at 25°C in quartz cuvette with 1 cm light path. The assay mixture contained (3-x) ml of tris buffer pH 8.0, 20 µl of sodium linoleate substrate and x ml of enzyme. After each addition, the mixture was stirred with a few strokes of plastic puddler. Absorption at 234 nm was recorded and the reaction rate was determined from the slope of the straight line portion of the curve. One unit of lipoxygenase is defined as a quantity of enzyme that generate one mole of conjugated diene per min under standard conditions. The extiniction coefficient for the linoleic acid product at 234 nm is 2.5×10^4 (Axelrod et al., 1981). Protein content was determined by the Hartree method (1972).

Results and Discussions

The inhibition of lipoxygenase activity by nicotine or rotenone insecticides was determined under the condition of varying concentrations ranging from 2.5×10^{-8} to 5×10^{-5} M , Fig. (1). Also the effect of preincubation period from simultaneously addition until reaching the zero order reaction level of inhibition was shown in Fig. (2). It is clear from the obtained data that, the inhibition increased with increasing nicotine or rotenone concentrations with I_{50} values equal 5×10^{-7} M and 2×10^{-7} M, respectively. Beside the inhibition of lipoxygenase activity was time dependent reaching its maximum inhibition after 30 min of preincubation period. Fig. (3), demonstrates the Lineweaver-Burk plot for lipoxygenase activity at different substrate concentrations in the absence and presence of 25 µM of nicotine (cause 44% inhibition) and rotenone (cause 38.5% inhibition) as inhibitors.

The data in Fig. (3) indicates that nicotine and rotenone inhibit the lipoxygenase enzyme by one K_m value which is equal to 0.66 µM. The data indicates that nicotine or rotenone inhibits the lipoxygenase enzyme by a non-competitively. This inhibition

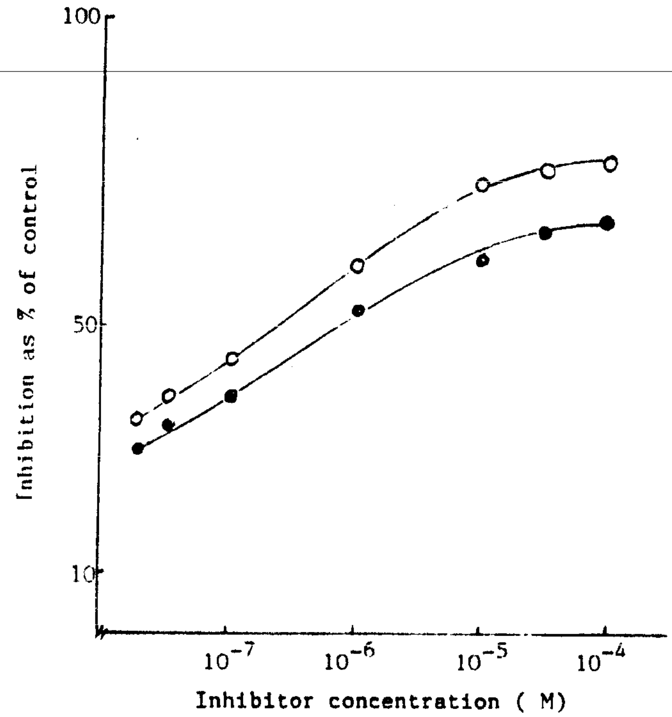

Fig. (1). Titration curve for lipoxygenase activity in presence of nicotine (●) and retenone (o) concentrations.

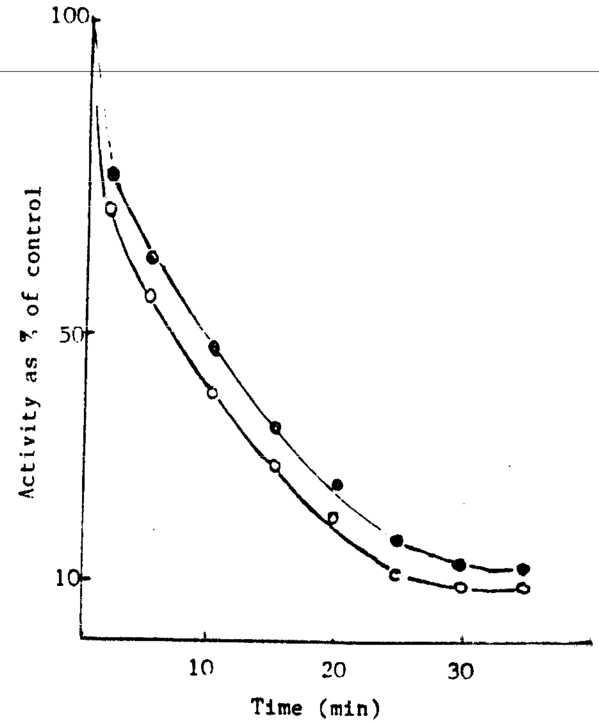

Fig. (2). Effect of preincubation period on nicotine (●) and rotenone (o) inhibitory effect on lipoxygenase enzyme activity effect may be due to blocking the oxidation of linoleic or by retarding the biradical product which is formed on the surface of the enzyme by accepting an electron from rotenone and forming H_2O_2 instead of formation of hydroperoxy-linoleate.

The data also shows that inhibition potential of rotenone is more than nicotine as clearly shown from the V_{max} calculations of lipoxygenase. Initial V_{max} was 30 µM/mg protein/min and then it become 20 µM/mg protein/min in presence of 25 µM of rotenone and 18.5 µM/mg protein/min in the presence of 25 µM nicotine concentration respectively.

The present study is the first one in reporting the inhibition of lipoxygenase enzyme by the insecticides, nicotine and rotenone. Rotenone is known to interfere with the respiratory chain by blocking the coupled oxidation of $NADH_2$ and reduction of cytochrome-b on the pyruvate side. On the other hand nicotine is a non-persistent, non-systemic contact insecticide. It is a nerve poison absorbed dermally or through inhalation. Cigarette smoking is known to be a major health risk for the development of cardiovascular diseases and pulmonary emphaysema. The biochemical mechanism by which smoking contributes to the genesis of these disorders are still incompletely understood. Recently, Mobley et al. (1987) reported that, the synthesis of 5-HETE (monohydroxyeicosatetranoic acid) and leukotriene B_4 was selectively inhibited by cigarette smoke exposure, whereas the formation of prostaglandin E_2 and thromboxane B_2 remained unchanged.

Prostaglandins, prostacyclin and thromboxane are products of the cyclooxygenase pathway, whereas leukotrienes are metabolites of the lipoxygenase pathway. These metabolites prossess vascoactive, bronchoactive, chemotactic and immunoregulatory properties and are implicated in maintaining vascular homeostasis, smooth muscle function, airway tones and inflammatory responses in the pulmonary system (Hammarastrom, 1983; Martin et al., 1984).

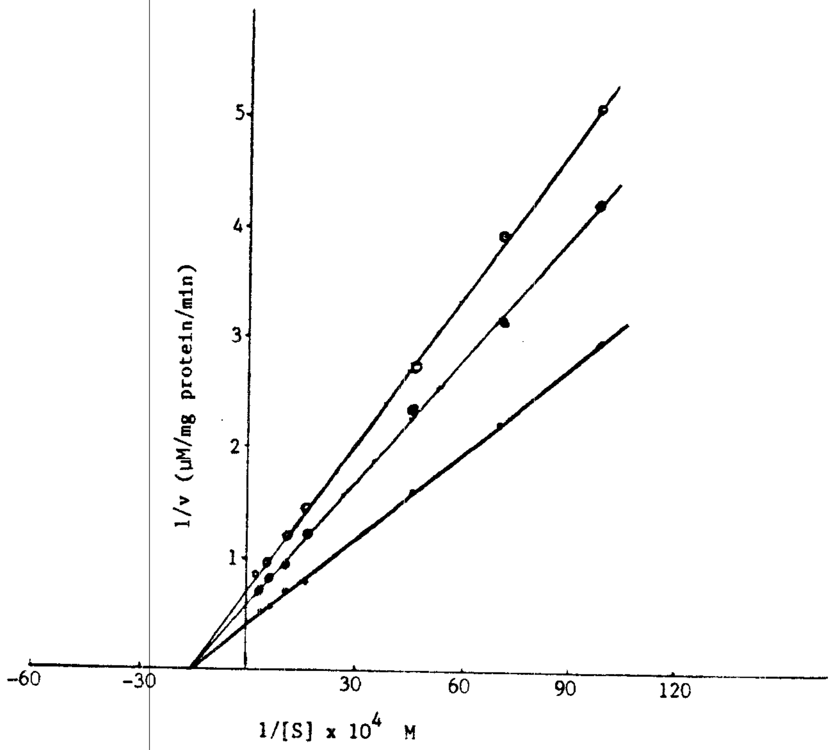

Fig. (3). Line-weaver-Burk plot for 1/v versus 1/[S] of lipoxygenase activity in absence (.) and presence of 25 µM of nicotine (•) and rotenone (o).

This study suggests that, the inhibition of lipoxygenase enzyme by cigarette smoking may be due to the impact of its nicotine content.

References

Axelrod, B.; T.M. Cheesbrough and S. Laakso (1981). Methods in Enzymology. Vol. 71, p. 441.

Baumann, J.; F.V. Bruchnausen and G.Wurm (1980). Prostaglandins, 20: 627.

Borg, D.C. (1970). Brook haven Series, Nat. Lab. BNL 50294, 90: 1-19.

Christopher, J.P.; E.K. Pistorws and B. Axelrod (1970). Biochem. Biophys. Acta, 198: 12.

Cohen, G. (1978). Phytochem. Phytobiology, 28: 699-675.

Dauillard, R. and E. Bergesn (1981). Physiol. Plant, 51: 335-338.

Eskin, N.A.M.; S. Grossman and A. Pinsky (1977). GRC-Crit. Rev. Food Sci. Nutr. 9: 1.

Fridovich, J. (1976). Oxygen radicals, hydrogen peroxide and oxygen + oxicity; In free radicals in biology (J.W. Pryor, ed.) Vol. 1, pp 239-277. Academic Press, N.Y. ISBN.

Hammarstrom, S. (1983). Ann. Rev. Biochem., 50: 355-377.

Hartree, E.F. (1972). Anal. Biochem., 48 (2): 422.

Martin, I.R.; L.C. Altman; R.K. Albert and W.R. Henderson (1984). Am. Rev. Respir. Dis., 129: 106-111.

Mobley, A.; H. Tanizawa; T. Iwanaga; C.L. Tai and H.H. Tai (1987). Biochimica et Biophysica Acta, 918: 115-119.

Siegel, M.T.; R.T. Mc Connell; N.A. Forter and Cuatrecasas (1980). Proc. Natl. Acad. Sci., USA, 77: 308.

Tappel, A.L.; W.O. Lundberg and P.D. Boyer (1953). Arch. Biochem. Biophys., 42: 293.

Vliegenthart, J.F.G. and G.A. Veldink (1982). In free radicals in biology. Vol. 5: 29-64. Academic Press, New York.

أنزيم الليبوكسى جينيز كهدف جديد لتأثير مبيدى النيكوتين والروتينون

نادر شاكـــر و ناديـــه شعبـــان

قسم وقاية النبات بكلية الزراعه و قسم الكيمياء الحيويه بكلية العلوم

جامعة الاسكندريــــــــــه

الملخص العربـــــى

تم دراسة نشاط انزيم الليبوكسى جينيز تحت الظروف المناسبه له الذى اظهر نشاط طبيعـــــــى معتمدا على تركيز البروتين الانزيمى ليعطى اعلى نشاط يساوى ٢٠ ميكرومول من مادة التفاعـــــــل متحلله لكل ١ مجم بروتين انزيمى لكل دقيقه .

أظهر النيكوتين قوة تثبيطيه لانزيم الليبوكسى جينيز معتمدا على تركيز المبيد المستخدم ليظهـــــر أن قمة التركيز الممثل لتثبيط ٥٠٪ من نشاط الانزيم هى ٥×١٠$^{-٧}$ مولر . كذلك قيم ثابت ميخائيـــل للانزيم فى وجود تركيز المبيد أو للانزيم بمفرده كانت ٦٦ر٠ ميكرومول فى تثبيط غير تنافس مع تركيز مادة التفاعل . وأعلى نشاط للانزيم فى وجود ٢٥ ميكرومول من تركيز المبيد كان ٥ر١٨ ميكرومول مـــــادة تفاعل محلله لكل ١ مجم بروتين انزيم لكل دقيقـــــه .

أوضحت النتائج أن الروتينون يثبط انزيم الليبوكسى جينيز بطريقه متناسبه مع زيادة التركيز بعـــــد عملية التحضين للبروتيـــــن الانزيمى وتركيز المبيد المستخدم وقد اظهرت النتائج أن تركيز المبيـــــــد المثبط لـ ٥٠٪ من النشاط الانزيمى كانت ٢×١٠$^{-٧}$مولر . يثبط الروتينون انزيم الليبوكسى جينـــــيز فى تثبيط غير تنافس مع مادة التفاعل بثوابت تثبيط مقدارها ٦٦ر ميكرومول كثابت ميخائيل وأعلـــــــى نشاط تثبيطى عند تركيز ٥ر٢×١٠$^{-٥}$ مولر كانت ٢٠ ميكروموول مادة تفاعل متحلله لكل ١ مجم بروتـــــين أنزيمى لكل دقيقـــــه .

التثبيط المتخصص والاختيارى لمبيدى النيكوتين والروتينون لانزيم الليبوكسى جينيز يظهر اهميـــــه خاصه ليس فقط لاستخدامهم لدراسة طرق تخليق الليكوترين ولكنه يعتبر هام للدراسات التكسكولوجيـــــه لهذه المجموعه من المبيدات .