International Journal of Applied and Natural Sciences (IJANS)
ISSN: 2319-4014
Vol.1, Issue 2 Nov 2012 59-67

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

PROPERTIES OF A SOLUBLE n-ALKANE MONOOXYGENASE FROM EXTRACTS OF CLADOSPORIUM RASINAE

[1]T. MEDHI, [2]S. KAR & [3]P. GOSWAMI

[1]Assistant Professor, Department of Molecular Biology and Biotechnology, Tezpur University, Napaam, Tezpur-784028, Assam, India

[2]Department of Agricultural and Food Engineering, Indian Institute of Technology, Kharagpur-721 302, West Bengal, India

[3]Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India

ABSTRACT

Cytochrome P450 oxidoreductase of fungui play diverse role in many essential processes of cellular metabolism. Hydrocarbon utilizing ascomycete Cladosporium rasinae catalyses initial oxidation of n-hexadecane by n alkane monooxygenases(AMO). The initial study indicates that the corresponding enzyme is present in soluble extracts of Cladosporium rasinae ATCC22712 obtained by centrifugation of crude extracts at 150 000g for 2 hours, catalyze the NAD(P)H and oxygen dependent oxidation of n-hexadecane. The constitutive enzyme system resides mainly in cytosol and functions in soluble form. It prefers NADH as electron donors to NADPH. Soluble n-alkane monooxygenase is a multicomponent enzyme not inhibited by many electron transport inhibitors and chelating agents excepting EDTA.

KEYWORDS: Cladosporium, Inhibitors, Monooxygenase, NADH, n-Hexadecane

INTRODUCTION

The study on microbial metabolism of hydrocarbon is stimulated due to its usefulness in producing important enzymes having applications in bioremediation technology and/or industrial production of secondary metabolites and other fine chemicals. The metabolism of highly water insoluble hydrocarbon is fairly understood in bacteria and in few species of yeast. However, the research on fungal metabolism of hydrocarbon is not gaining momentum due to the hindrances like slow growth of fungi on hydrocarbon substrate and difficulties in preparing pure sub-cellular organelles from hydrocarbon grown cells (Carson and Cooney 1990; Lindley 1995; Lindley et al. 1996; Valenciano et al. 1996).

The only filamentous fungus on which little progress is made till now is Cladosporium resinae. Apart from the proposed monoterminal metabolic pathway based on the intermediate product analysis, the study so far revealed its NADH dependence for activity (Walker and Cooney, 1973a) and the constitutive nature of the enzyme (Walker and Cooney, 1973b).

The other related findings are the cellular accumulation of substrate hydrocarbons (Cooney et al., 1980) and proliferation of microbodies in n-alkane grown cells (Smucker and Cooney 1981). It has previously been shown that the enzyme is mainly located in the cytoplasm (Goswami and Cooney 1999).

Recently a cytochrome P450 monooxygenase isolated from white rot bsidiomycete Phanerochaete chrysosporium was heterolgously expressed in E coli and purified in active form(Ichinos and Wariishi,, 2012).

The present paper reports on preliminary findings about the properties of a soluble n-alkane monooxygenase isolated from Cladosporium rasinae ATCC 22712.

MATERIALS AND METHODS

The Organism and Culture Conditions

Culture of Cladosporium rasinae ATCC 22712 was maintained on Sabouraud Dextrose Agar (Hi-Media) slants, which were inoculated at 30^{o} C and then stored at 4^{o} C. The basal medium consisted of Bushnell and Haas salt solution (Bushnell and Haas, 1941), supplemented with 0.1% (w/v) yeast extract. The pH was adjusted to 5.8 with HCl, and 50 ml volumes were dispensed into 250 ml Erlenmeyer flasks. The salt solution was then overlaid with hexadecane to a final concentration of 2%(v/v). The preparation was then sterilized by autoclaving. A loopful of cells from a slant was used as inoculums for static cultures incubated at 28^{o} C. Cells were harvested after 8 days.

Growth and Preparation of Soluble Extracts

Mycelial cultures were grown in Bushnell and Haas Medium as mentioned above and were harvested after 8 days. Cells were collected on 20 μm aperture nylon mesh by vacuum filtration and washed three times on the filter with Bushnell and Haas Medium. Cells were then washed twice with hexane, a process that removes extra cellular hydrocarbons without removing the intracellular hydrocarbons followed by three additional washes.

After recording wet weight, the mycelial mass is dipped in liquid nitrogen for 1-3 seconds, the cell mass was then suspended in an extraction buffer of composition (g 20 mM potassium phosphate buffer 100ml^{-1}), 11.98g sucrose, 100μL of 1mM DTT solution and 100μL of 1mM EGTA solution. The crude mycelial extracts were prepared by two passages of the suspension through French Pressure Cell (Aminco, Silver Spring, Maryland) at 137 MPa(20,000 lbinch^{-2}). This method was superior to grinding with sand. Grinding with sand was carried out in a mortar pestle at 4^{o} C after treating the cell mass similarly with liquid nitrogen. The process of grinding was repeated after centrifugation until there was leftover activity. The same was confirmed by examining the treated mycelial mass under microscope. The homogenate was centrifuged in a Sorvall 80 ultra centrifuge at 38,000g for 30 minutes at 4^{o}C. The supernatant was designated as S_{38} used as cell extract. The S_{38} fraction was subsequently centrifuged at 150 000g for 2 hours to give a clear yellowish supernatant (S_{150}).

Preparation of Cytochrome C Fraction

A preparation of cytochrome C was obtained by concentrating a 20 ml of preparation of S_{38}(described above) by ultrafitration by pressure, with an Amicon PM10 membrane(Amicon, High Wycombe, Bucks, U.K.) down to 5ml.

Protein content

The protein concentration was determined with the Folin- Ciocalteau reagent (Lowry et al., 1951), with dried crystalline bovine plasma albumin as standard.

Enzyme Assays

Glucose-P-dehydrogenase(EC 1.1.1.49) was measured by monitoring the production of NADPH at 340 nm(ξ = 6.22 mM^{-1}cm^{-1}) following a modification of Kornberg and Horecker(1955). The assay mixture contained 2.2ml H_2O, 0.1 ml 0.05M NADP, 0.3ml 0.1 M glycylglycine buffer pH 7.5, 0.2 ml 0.01 M MgCl2, 0.1 ml 0.5 M glucose-6-phosphate and 0.1 ml cell extract. Controls were run for endogenous NADPH production.

NADH dependent n-alkane monooxygenase activity was assayed by measuring the substrate dependent oxygen consumption. The reaction mixture used for the assay of n-alkane monooxygenase activity consists of 4.0 μl n-hexadecane, 3 ml of 50 mM citrate phosphate buffer of pH 4.5, and 0.5mg Plysurf A210G (Diachi Kogyo Seiyaku, Kyoto Japan, supplied by Prof. Nobuo Kato, Kyoto University Japan), 1 mM NADH and 0.2 ml enzyme. n-Hexadecane and Plysurf

A210G were added to the buffer and heated in a boiling water bath for 5 min and then homogenized by sonication at 20KHZ(150W) for 1 min in a Labsonic U sonicator. The substrate dependent oxygen consumption is measured in a closed reaction vessel in a Model 53 biological oxygen monitor (Yellow Springs Instrument Co., Yellow Springs Ohio, USA) fitted with a Clarke type oxygen electrode. The oxygen probe was calibrated by using oxygen consumption in a Xanthine Xanthine oxidase system as described by Billiar et al. (1970). The same reaction mixture and temperature were used as stated earlier. After homogenizing the hydrocarbon and PlysurfA21OG in buffer the homogenate along with an appropriate amount of enzyme was poured into a magnetically stirred airtight chamber. NADH was injected in to the chamber when the recorder showed a consistent base line. By running a control where instead of enzyme solution the corresponding control was added to eliminate endogenous oxygen consumption. Activity was expressed as the amount of enzyme needed to consume 1μmol of oxygen (O_2) per min.

RESULTS & CONCLUSIONS

Differential centrifugation of broken cell suspensions of Cladosporium rasinae has yielded cell-free supernatant fractions which catalyses the oxidation of n-hexadecane and other alkane derivatives (Table1) depending on preparations. The activity of n-alkane hydoxylase was routinely assayed by the NADH dependent consumption of O_2. n-Alkane monooxygenase activities associated with cell free extracts of Cladosporium rasinae ATCC 22712, prepared either by abrasion (mortar pestle) or by two passages through French pressure cell are shown in Table 2. Higher specific activities were observed in French pressure cell, probably due to higher recovery for such extracts compared to abrasion. Since under the conditions used for cell extract preparation, the enzyme gradually losses activity. Only n-hexadecane oxidation showed a requirement for NADH.

Fractions prepared by centrifugation at 38 000x g for 30 min appears dark brown cell-free preparations. Further fractionation of the supernatant1 by one differential sedimentation at 150 000 x g for 2h, yields a clear light yellowish cell free preparation. Pellets obtained after centrifugation at 38,000xg and 150,000xg contain smaller membrane fragments with no demonstrable NADH dependent n-alkane monooxygenase activity. The distribution of NADH dependent n-alkane monooxygenase activity is given in Table 3. After centrifuging, practically all n-alkane monooxygenase activity was recovered in supernatant fraction. The particulate and supernatant fractions were prepared by centrifugation at 150 000xg exactly as described in methods and materials. An initial centrifugation of 38 000g for 30 min was used routinely to remove unbroken cells because of viscous nature of the preparations. As with the most hydroxylase enzymes, the AMO is also not stable at room temperature. When stored in -75^{o}C,the supernatant (S_{150}) fraction retained its full activity for several months and at 0^{o}C retained 80 – 100% activity for 24hrs. Efforts are on to purify the enzyme system to determine the in-situ physico-chemical environment for catalysis of AMO and kinetic behaviors of the enzyme system.

Crude extracts of Cladosporium rasinae oxidizes n-hexadecane in presence of NADH and O_2. In our study, the light yellowish supernatant obtained after centrifugation at 150 000xg, catalyses hexadecane oxidation. This indirectly indicates the presence of AMO of Cladosporium rasinae in cytoplasm. The cytochrome C-450 in yeast and higher eucaryotic cells is reported to be microsomal (Gallo et. al., 1972). However the occurrence of the AMO activity of Cladosporium .resinae in the cytoplasm (Goswami & Cooney 1999) apparently ruled out the involvement of cytochrome C-450 in the catalytic activity of AMO. Hence further investigation is needed to elucidate the nature of the electron carrier and mechanism of electron transport during AMO catalysis of filamentous fungi.

Although not conclusive, this study shows properties of hydroxylase enzyme distinct from the enzymes reported in yeasts and mammalian systems. Here some important properties of the soluble n-alkane monooxygenase activity in S_{150} were investigated by using substrate dependent oxygen consumption as the assay method. Unless specified otherwise the

results mentioned were obtained by using the same method. From the limited range of electron donors and acceptors tested (Table 4) hexadecane oxidation shows a specific requirements for both NADH and oxygen. Activity was highest at pH 4.0 - 4.5. Table 3 shows NADH (10mM), NADPH (10mM) or and NADPH generating system consisting of $NADP^+$(4mM), glucose -6-Phosphate (8mM) and endogenous glucose -6-Phosphate dehydrogenase (EC 1.1.1.49)(Cox et al., 1982) were necessary for activity. Highest activities were noted with NADH and or the NADH generating system, NADPH was 80% as effective as NADH at the concentrations used. There was no synergistic effect on n-alkane oxidation rate by Cladosporium rasinae, as was observed in Candida tropicalis(Duppel et al., 1973)when NADH and NADPH were used in combination in the reaction mixture. In yeast, hydroxylases system is found to be specific for NADPH.

Hexadecanol, ethanol, sodium ascorbate all did not support n-alkane monooxygenase activity. The electron donors required for methane monooxygenase activity of Methylococcus capsulatus bath, Methylomonas methanica and Methylosinus trichosporium are well documented(Colby et al., 1975, Tonge et al., 1975). In mammalian microsomal prepareation, Sodium chlorite and Sodium periodate have been shown to support steroid hydroxylation by replacing NADPH in the cytochrome P –450 system (Hrycay et al., 1975).

A range of potential electron transport inhibitors was tested on n-hexadecane oxidation by S_{150} of Cladosporium rasinae. The enzyme system is not inhibited by relatively high concentration of KCN (1 mM) and amytal. Exogenous cytochrome C prepared from extracts of Cladosporium rasinae do not stimulate or restore activity to various n-alkane monooxygenase preparations. This fact and the inhibitor pattern, emphasizes that cytochrome is not directly involved in the n-alkane monooxygenase system as has been observed in n-alkane utilizing yeasts (Fukui and Tanaka, 1981, Lebeault et al., 1971). In bacteria also the occurrence of cytochrome P-450 in hydroxylating enzyme system is prominent (Cardini et al., 1968, Sheldon et al., 1990). Amytal (4mM) prevented NADH mediated but not ascorbate mediated methane oxidation by the particulate methane monooxygenase in Methylosinus trichosporium (Tonge et al., 1975). Similar observation was made by Ribbons (1975) in case of Methylococcus capsulatus(Texas). For NADH dependent n-alkane oxidation in Cladosporium rasinae the observations suggest NADH is not an immediate electron donor in the transport process. The results obtained with the soluble extracts of Cladosporium rasinae are given in Table 5. The weak inhibition patterns for a few of them suggest that the electron transport chain may not be directly involved in the passage of electron from NADH to n-alkane monooxygenases in case of filamentous fungus. Considerable inhibition by EDTA suggests involvement of metals in enzyme activity. However further studies with specific metal chelating agents will be required to see its functional roles of metal ions. Oxidation of toluene by ◂Cladosporium▸ sphaerospermum is initially catalyzed by toluene ◂monooxygenase▸ Addition of toluene to a glucose-pregrown culture of C. sphaerospermum induces TOMO activity. The corresponding is microsomal enzyme and needs NADPH and O_2 to oxidize toluene and glycerol, EDTA, DTT, and PMSF for stabilization. TOMO activity was maximal at 35 °C and pH 7.5 and was inhibited by carbon monoxide, Metyrapone, and cytochrome c(Luykx D M A M et. al. 2003). Vatsayan P et. al. (2008) determined cytochrome P450 (CYP) monooxygenase activities with different category of substrates namely, alkanes, alkane derivatives, alcohols, aromatic compounds, organic solvents, and steroids in the cells of Aspergillus terreus.

Apart from, the observations with inhibition pattern and electron donor study; the critical aspects to be considered further are constitutive nature of the enzyme, apparent non-involvement of cytochrome, instability and physicochemical environment of catalysis. It should be noticed here that like the mammals and yeasts the oxidation pathway is not microsomal here.

When n-alkane monooxygenase activity was plotted as a function of amount of soluble fraction present, a non-linear relationship was obtained (Fig 1). This suggests that the enzyme is composed of more than one component. This was

confirmed by resolution of S_{150} fraction in to at least two components by passing through a column of DEAE Cellulose. Neither the eluate nor materials retained by the column was active when tested separately, but activity was restored fully by combining both of them.

ACKNOWLEDGEMENTS

The Council for Scientific and Industrial Research (CSIR), Government of India supports this work and this is gratefully acknowledged. The authors are grateful to Dr. S. Sengupta, Indian Institute of Chemical Biology (IICB), Calcutta and Dr. Pradosh Roy, Bose Institute, Calcutta for their help in performing some experiments in their laboratories.

REFERENCES

1. Billiar, R.B., Knappenberger, M., and Little, B. 1970. Xanthine oxidase for calibration of the oxygen electrode apparatus. Anal. Biochem. 36: 101 104
2. Bushnell, L.D., and Haas, H.F., 1941. The utilisation of certain hydrocarbons by microorganisms. J. Bacteriol. 41: 653 673.
3. Cardini, G., and Jurtsluk. P., 1968. Cytoplasm P-450 involvement in the oxidation of n-octane by cell free extracts of Corynebacterium sp. Strain 7EIC. J. Biol. Chem. 243: 6071-6072.
4. Carson, D.B., and Cooney, J.J. 1988. Spheroplast formation and partial purification of microbodies of hydrocarbon-grown cells of Cladosporium rasinae. J. Ind. Microbiol. 3:111-117
5. Carson, D.B., and Cooney, J.J. 1990. Microbodies in fungi: a review. J. Ind. Microbiol. 6:1-18
6. Colby, J., Stirling, D.I., and Dalton, H. 1977. The soluble methane mono-oxygenase of Mythylococcus capsulatus(Bath). Biochem. J. 165: 395-402
7. Cooney, J.J., Siporin, C., and Smucker, R.A. 1980. Physiological and cytological responses to hydrocarbons by the hydrocarbon using fungus Cladosporium rasinae. Bot. Mar. 23: 227-232
8. Cox, C., Camus, P., Buret, J., and Duvivier, J. 1982 An enzymatic cycling procedure for NAD+ using irreversible reaction with NAD+ peroxidase. Anal. Biochem. 119: 185-193
9. Fukui, A., and Tanaka, A. 1981. Metabolism of alkanes by yeast. Adv. Biochem. Engg. 19: 217-237
10. Gallo, M., Bertrand, J.C., Roche, B., and Azoulay, E. 1972. Alkane oxidation in Candida tropicalis. Biochim. Biophys. Acta. 296:624-639
11. Goswami, P., and Cooney, J.J., 1999. Subcellular location of enzymes involved in oxidation of n-alkane by Cladosporium rasinae. Appl. Microbiol. Biotechnol. 51: 860-864.
12. Horecker, B. L., and Kornberg, A. 1948. The extinction coefficient of the reduced band of pyrimidine nucleotide. J. Biol. Chem, 175: 385 390.
13. Hrycay, E.G., Gustaffson, J.A., Ingraham-Sundberg, M., and Ernster, L. 1975. Sodium periodate, sodium chlorite and organic hydroperoxide as hydroxylating agents in hepatic microsomal steroid hydroxylation reactions catalysed by cytochrome p-450.FEBS. Lett. 56: 161-165

14. Ichinose, H., and Wariishi H. 2012, Heterologous expression and mechanistic investigation of a fungal cytochrome P450(CYP5150A2): Involvement of alternative redox partner. Arch of Biochem and Biophys, 518: 8-15

15. Katagiri, M., B.N. Ganguli., and I.C. Gunsalus., 1968. A soluble cytochrome P-450 functional in methylene hydroxylation. J.Biol Chem. 243,3543-3545.

16. Klug, M.J., and A.J. Markovetz, 1967. Degradation of hydrocarbons by members of genus

17. Candida II: Oxidation of n-alkanes by Candida lypolytica. J. Bacteriol. 93. 1847-1852

18. Klug, M.J., and A.J. Markovetz, 1967. Degradation of hydrocarbons by members of genus Candida III: Oxidation of n-alkanes by Candida lypolytica. J. Bacteriol. 96. 1115-1123.

19. Kornberg, A., and Horecker, B.L., 1955. Glucose-6-phosphate dehydrogenase. Methods. Enzymol. 1: 323- 327

20. Lebeault, J..M., Code, E.T., Conn, M.J., 1971. Fatty acid and hydrocarbon hydroxylation in yeasts: role of cytochrome p-450 in Candida tropicalis. Biochem. Biophys.Res.Comm.42: 413-419.

21. Lindley, D.L., 1995. Bioconversion and biodegradation of aliphatic hydrocarbons Can. J. Bot. 73[Suppl 1]: S1934-S1042

22. Lindley, N.D., Pedley, J.F., Kay, S.P., Heydeman, M.T., 1996. The metabolism of yeasts and filamentous fungi which degrade hydrocarbon fuels. Int. Biodeterior. 22: 281-287

23. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265-275

24. Luykx D M A M, Prenafeta-Boldu, F X, deBont J A M 2003 Toluene monooxygenase from the fungus Cladosporium sphaerospermum Biochem. and Biophys. Res. Comm, 312(2) 373-379

25. Ribbons, D.W., 1975 Oxidation of C1 compounds by particulate fractions from Methylococcus capsulatus : Distribution and properties of methane dependent reduced nicotinamide adenine dinucleotide oxidase(methane hydroxylases). J. Bact. 122: 1351- 1363

26. Sheldon, W.M., Andreas, G.K., 1990 Hydrocarbon monooxygenase system of Pseudomonas oleovorans. Methods in Enzymolozy, 188: 3-9.

27. Smucker, R.A., Cooney, J.J., 1981. Cytological response of Cladosporium rasinae when shifted from glucose to hydrocarbon medium. Can. J. Microbiol. 27: 1209-1218

28. Stirling, D.I., Dalton, H., 1979. Properties of Methane monooxygenase from extracts of Methylosinus trichosporium OB3b and evidence for its similarity to the enzyme from Methylococcus capsulatus(Bath). Eur. J. Biochem.. 96: 205-212

29. Tonge, G.M., Harrison, D.E.F., Knowles, C..J., Higgins, I.J., 1975 Properties and partial purification of the methane oxidising enzyme system from Methylosinus trichosporium.. FEBS. Lett. 58: 293-299

30. Valenciano, S., DeLucas J.R., Pedregosa, A., Monistrol, I.F., and Laborda, F., 1996. Induction of β-oxidation enzymes and microbody proliferation in Aspergillus nidulans. Arch. Microbiol. 166:336-341

31. Vatsyayan, P. Kumar,K, Goswami, P and Goswami P., 2008 Broad substrate cytochrome P450 monooxygenase activity in the cells of Aspergillus terreus MTCC 6324 Biresource Technology, 99(1) 68-75

32. Walker, J.D., Cooney, J.J., 1973a Pathway of n alkane oxidation in Cladosporium rasinae. J. Bacteiol. 115: 635 639.

33. Walker, J.D., and Cooney, J.J., 1973b. Oxidation of n alkanes by Cladosporium rasinae. Can. J. Microbiol.. 19:1325 1330.

APPENDICES

Table 1: Oxidative Activities of a Supernatant Fraction from Cladosporium Rasinae [a]

Substrate	Oxygen consumption (nmol/min/mg of protein)
None	0.5
NADH	ND
n-hexadecane	3
n-hexadecane +NADH	9
n-hexadecanol	10
n-hexanaldehyde	12
n-hexadecanoate	ND
n-hexadecanoate + NAD	**ND**

[a] Each reaction mixtures contained: 20 mM citrate phosphate buffer, air saturated (2.7 ml); supernatant fraction 2(1000 µl containing 0.4 mg of protein) ; and 25 mM NADH (30 µl) as indicated.

Hydrocarbons were added as saturated solutions in the buffer (0.3 ml). For alcohol, aldehyde, and acid substrates additions of 100 mM solutions (0.2 ml) were made. Temperature, 30^{o} C.

Table 2: n-Alkane Monoxygenase Activity in Cell Free Extract Preparations

Fraction	Activity in fraction obtained by	
	Morter pestle	French Pressure Cell
	munits.min^{-1}mg protein^{-1}	
Cell free Extract	90	106
Supernatant	105	140
Particulate	10	7
Supernatant +Particulate	109	140

Cell free Extract is prepared after centrifugation at 38 000g for 30 min.

Supernatantis prepared after centrifugation at 150 000g for 2 hrs.

Table 3: Distribution of NADH Stimulated n-Alkane Monooxygenase Activity [a]

Cell Fraction	Oxidation rates (munits/min/mg protein)		
	Endogenous	+NADH	+NADH + n-hexadecane
Washed cell suspension	25	ND[b]	36.4[c]
Disintegrated cell suspension	ND	ND	9.8
Supernatant 1	1.5	8	13.0
Supernatant 2	ND	18	14.0
Pellet 1	ND	ND	1.2
Pellet 2	ND	ND	1.0

[a] Reaction mixtures contained: 50 mM citrate phosphate buffer, pH 5.5, air-saturated (2.7 ml) or 2.5 ml plus 0.5 mg PlysurfA210G, 1mM NADH and 0.2 ml cell fraction. Temperature, 30°C. Each particulate fraction was resuspended in buffer to give the original volume of the cell suspension.

[b] ND, Not determined.

[c] Activity in the absence of NADH but the presence of n-hexadecane.

Table 4: Effect of Different Potential Electron Donors and Hydroxylating Agents

Test Compound	Specific Activity (munits/mg of protein)	
	Oxygen Consumption	Hexadecane Disappearance
NADH (10mM)	230	10
NADPH (10mM)	180	7
NADH (2mM) + NADPH (10mM)	220	10
NADH (10mM)+Catalase(50units)	260	12
Xanthine(5mM)+Xanthine oxidase (1 unit)	0	0
Glucose(1mM)+Glucose oxidase(1 unit)	0	0
H_2O_2(5mM)	20	ND

ND = not determined

Table 5: Effect of Electron-Transport Inhibitors on n-Alkane Monooxygenases Activity

Inhibitor	Concentration (mM)	Inhibition (%)
KCN	1	0
NaN3	5	0
EDTA	1	5
EDTA	5	80
EDTA	1	0
Ethyl carbamate	1	15
ActinomycinA	1	0
Rotenone	1	3
Amytal	1	0
Oligomycin	1	0
Acriflavin	1	0

The various compounds were preincubated for 15 min at 0^0C with all the components present except n-hexadecane and NADH. The reaction mixture is then brought to 30^0C and activity was determined by the standard procedure.The protein content of S_{150} was .0.74 mg ml^{-1}.

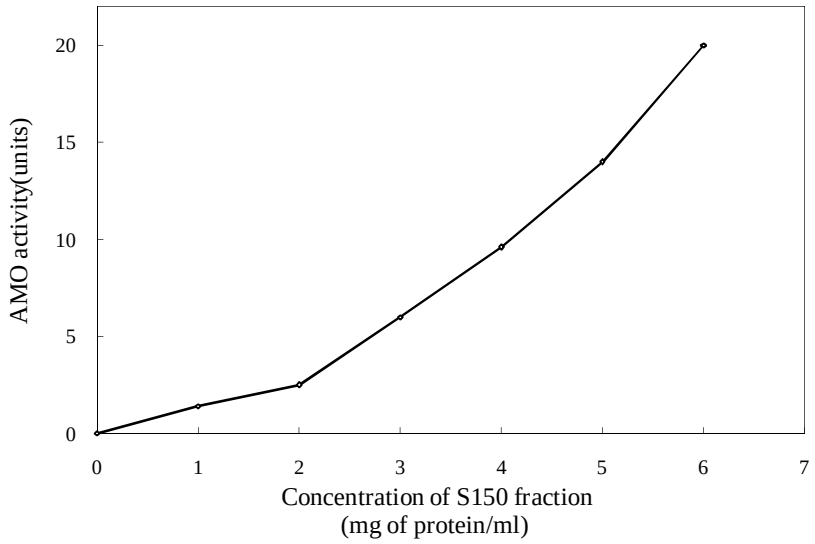

Fig.1: Non-Linear Relatioship Between n-Alkane Monooxygenase Activity and Extract Protein Concentration Activity Determined By Measuring Oxygen Consumption (β)