Uses of peroxidases enzymes

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Peroxidases are oxidoreductases produced by number of plants and microorganisms. Reduction of Peroxidases in presence of electron donating substrate makes Peroxidases useful in many commercial applications. Peroxidase enzymes such as manganese and lignin peroxidase both associated with lignin degradation, which is successfully used for biobleaching and biopulping in the paper industry, and can produce oxidative break down of synthetic azo dyes. Peroxidase based biosensors are widely used in analytical systems for determinations of hydrogen peroxide, and co immobilized with hydrogen peroxide producing enzyme which are used for determination of glucose, alcohols, glutamate and choline. Peroxidase has also been used for practical analytical applications in diagnostic kits, such as quantitation of uric acid, glucose, lactose, cholesterol, etc. Enzyme linked immunorbent assay (ELISA) tests on which peroxidase is the most common enzyme used for labeling an antibody, are a sample and reliable way of detecting toxins, pathogens, cancer risk in bladder and prostate. Peroxidase is potential has potential for soil detoxification while Horseradish peroxidase is widely used in waste water treatment in order to remove the pheonlic, cresol and chlorinated phenols. With uses of peroxidases enzymes for commercial uses in various fields and there potential of large scale production also gained major importance as the main source of horseradish peroxidase is produced from horseradish, latest research shows many plant and microbial source for the production of peroxidases enzymes.

Key words: Immunoassays, peroxidase, diagnostic kits, biosensors, bioremediation.

Introduction:

The term peroxidase enzyme represents group of specific enzymes, divided into plant and mammalian Peroxidases. Most Peroxidases are heam proteins and contain iron (3) protoporphyrin 1x (ferriprotoporphyrin 1x), as prosthetic group (Carlos et al., 2004) they have a molecular weight ranging from 30000 to 150 000 da. Peroxidase activity has been identified in plants, animals and micro organisms. In plants they play a major role in lignifications process (wakamatsu and Takahama, 1993) and in the mechanism of defense in physically or infected tissues (Biles and Martin, 1993). Peroxidases catalyze the oxidation of a wide variety of substrates, using H2O2 or other peroxides. The peroxidase catalytic cycle involves distinct intermediate enzyme forms (Wong, 1995). In the initial step the native ferric enzyme is oxidized by hydrogen peroxide to form an unstable intermediate called compound I (CoI), which has a heme structure of FeIV= Oporphyrin π-cation radical, and the consequent reduction of peroxide to water. Then, CoI oxidizes an electron donor substrate to give compound II (CoII) (same oxyferryl structure, but protonated), releasing a free radical. CoII is further reduced by a second substrate molecule regenerating the iron (III) state and producing another free radical.

(Carlos et al., 2004).

Reduction of peroxides at the expense of electron donating substrates, make Peroxidases useful in number of industrial and analytical applications. peroxidase is the most well suited enzyme for the preparation of enzyme conjugated antibodies which are used in enzyme linked immunosorbent assay test (ELISA) , due to its ability to yield chromogenic products at low

concentration (Krell, 1991). Peroxidase coupled with other enzymes in polyenzymatic systems producing hydrogen peroxide is also used in the determination of many compounds, such as glucose in blood. Because of the oxidative nature of Peroxidases, there are several areas where it could replace current chemical oxidant techniques. Horseradish (Armorica rusticana L.) roots contain peroxidases currently used for the commercial production of reagents for clinical purposes and enzyme immunoassays (Agostini et a., 1996). But research shows there are many other sources for the production of peroxidase enzymes. Agostini et al., 1996 has successfully produced peroxidase enzymes from hairy roots of Brassica napus. These are successfully used in commercial kits. Soudek et al., were optimized in production of peroxidase by tissue culture from horseradish.

COMMERCIAL USE OF PEROXIDASE ENZYMES

Application in analysis and diagnostic kits

Galactosemia is genetically inherited metabolic disorder characterized by an inability of the body to utilize galactose (Jie et al., 2002) galactose is a type of food sugar found mainly in dairy products and produced within the human body. The main source of galactose is milk products. Milk contains sugar called lactose (disaccharide sugar), which will hydrolyzed into glucose and galactose (monosaccharide sugars) (Sharma et al., 2004) glucose is utilized as source of energy. In presence of enzymes galactokinase, galactose -1-phosphate uridyl transferase and galactose 6- phosphate epimerase galactose ifs further metabolized (Petry and Reichardt, 1998) deficiency of any of these enzymes leads to galactosemia which results in cataracts in infants, liver disease and kidney problems, if galactosemia is not treated.

High levels of galactose and galactose 1-phosphate can cause brain damage may lead to death (Sharma et al., 2004). It is very important for the people with galactosemia to avoid food contain galactose/lactose in the diet.

Sharma et al developed an immobilized enzyme based Biostrip for estimation of galactose in food products. The enzymatic method for determination of galactose is based on oxidation of galactose by galactose oxidase to form D-galacto-hexodialdose and hydrogen peroxide . In presence of peroxidase form colored complex with o-dianisidine or o-tolidine (S. K. Sharma et al .,). the Biostrip was dipped in milk or a milk product and color developed from an added chromogen . The test is mostly used in dairies, hospitals, and homes.

Principle of the Biostrip

Detection of cholesterol level in serum

In human monitoring cholesterol level in serum is very important, increase are decrease in level of cholesterol in serum is associated with diseases (Hirany, Li, and jialal, 1997), thus there is need for analysis , Malik and Pundir (2002) developed a new diagnostic kit. In this method cholesterol ester is hydrolyzed by cholesterol esterase to free fatty acids and cholesterol, which is oxidized by cholesterol oxidized to cholestenone and H2O2. H2O2 Is determined using HRP the following equation . where the resultant quinoneimine is measured at 520 nm . This method is most employed in commercial kits (Mohsina et al., 2009).

HRP is used in identifying bladder and prostate cancer by detecting 8-hydroxydeoxyguanosine and its analogs in urine. (Carlos et al., 2004)

In the latest research by J Tupper (2010) reported HRP is used in gene therapy of cancer. Gene -directed enzyme prodrug therapy is a form of targeted cancer therapy, in which an enzyme is used to convert a non toxic prodrug to a cytotoxin within the tumor . Horse radish peroxidase, is able to convert the indole prodrugs indole-3-acetic acid (IAA) and the halogenated derivative 5-bromo-iAA (5br-IAA) to toxic agents able to induce cell kill in vitro.

Peroxidase immunoassays

ELISA enzyme linked immunosorbent assays are tests designed to detect antigens or antibodies by producing an enzyme triggered change of color. To this an enzyme labeled antibody specific to antigen is needed, as well as chromogenic substrate, which in presence of enzyme changes color. The amount of developed color is directly proportional to the amount of antigen in the test specimen.

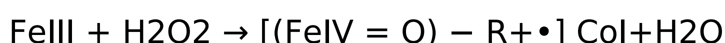
HRP is probably the most common enzyme used as an enzyme labeled antibody in the enzyme immunoassays. Peroxidase based ELISA test are used for labeling an antibody, is used for screening of monoclonal antibodies against mycotoxins. (Kawamura et al., 1989). Mycotoxins are dangerous toxins released by fungi. Mycotoxins are mostly found in cereals and their detection is necessary to avoid risk of consumption.

Peroxidase biosensors

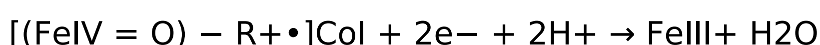
One field that offers great potential for peroxidase application is that comprising electrochemical biosensors. Recently, peroxidase based electrodes have had widespread use in analytical systems for determination

of hydrogen peroxide and organic hydroperoxides (Jia et al., 2002). When co-immobilized with a hydrogen peroxide producing enzyme, they may be exploited for determination of glucose, alcohols, glutamate, choline (Ruzgas et al., 1996).

Peroxidase first catalytic cycle involves reaction of the active site with hydrogen peroxide



Then, CoI oxidizes a substrate to give a substrate radical and CoII, which is reduced by a second substrate molecule, regenerating the native ferric enzyme. When an electrode substitutes the electron donor substrate in a common peroxide reaction cycle, the process is denominated as direct electron transfer (Freire et al., 2003). Enzymes immobilized on an electrode can be oxidized by hydrogen peroxide (equation 2) and then reduced by electrons provided by an electrode



When an electron donor (A) is present in a peroxidase-electrode system, the direct process ca occur simultaneously, with reduction of the oxidized donor A• by the electrode (Liu and Ju, 2002;

During direct electron transfer, electrons act as the second substrate for the enzymatic reaction, resulting in a shift of the electrode potential, with the measured current being proportional to the H_2O_2 concentration (Everse et

al., 1991). This technique can also be used to quantify other metabolites, especially combined with another oxidase enzyme.

Peroxidase can also interact with an electrode by mediated electron transfer, where a mediator (an electron donor, A) is transporting the electrons between the enzyme and the electrode. In this system, the enzymatically oxidized donor (A•) is thus electrochemically reduced by the electrode.

Application in the paper pulp industries

The biopulping is a process where the extra cellular enzymes produced by a white rot fungi remain absorbed on the wood chips degrading lignin . after pulping process, about 10% of the lignin which is responsible for characteristic brown color (carols regalado et al.,). lignin can be enzymatically degraded using enzymes like manganese Peroxidase (Hatakkaet al.,) . thus manganese Peroxidase is potentially use full in biobleaching (Carlos regalado et al.,)

Decolorization of synthetic dyes

Dyes are used extensively for paper printing , color photograph , textile dyeing and as additive in petroleum industries . they have synthetic origin and complex molecular structure . the used synthetic dyes are discharged in to waste water causing environmental problems . It is very important to treat dyes before discharging . Bhunia et al ., showed the horse radish peroxidase HRP can be effective in degrading and precipitating industrially important azo dyes such as remazol and cibacron red.

Deodorization of swine manure:

Pork meat production is considered as one of the major food industries . In practice pork farms face major challenge in storage and processing of large amount of swine manure, which becomes a serious management and environmental issue, swine manure consist of concentrated organic materials, decomposition of which result in the production of malodorous, volatile low molecular weight compounds such as volatile fatty acids and aromatic compounds such as phenol, indoles and cresols as well ammonia and hydrogen sulfide.(fen xia ye et al., 2009) which are odorant compounds. Elevated odor level in buildings can reduce live stock growth rate and increase the out breaks of infections and adversely affect farm workers.

HRP has been proven as an effective alternative for deodorization of manures. Minaced horseradish with calcium peroxidase reduced concentration of phenols by 70% and volatile fatty acids for 45% (Gover et al., 2007). A 100% reduction in the concentration of phenol odorants without reoccurrence within 72% h was achieved by using HRP (Gover et al., 2005).

Commercial uses of lactoperoxidase:

Dairy products provide high quality protein rich food for human consumption. However raw milk is very perishable food stuff special measure are taken in order to stop the growth of microorganisms, while transporting milk from farmers to dairy industries over a longer distances. To solve this problem storage for longer time naturally occurring antimicrobial system in milk know as lactoperoxidase system, which is activated in milk by increasing the concentration of thiocyanate and hydrogen peroxide. The lactoperoxidase (LP) system consists of three components: Lactoperoxidase, thiocyanate, and

hydrogen peroxide (H_2O_2) and it is active only in the presence of all these three components. Lactoperoxidase catalyses the oxidation of thiocyanate (SCN^-) by H_2O_2 and generates intermediate products with antibacterial properties. These products have a broad spectrum of antimicrobial effects against bacteria, fungi and viruses (de Wit and van Hooydonk, 1996).

Guthrie (1992) reported the application of the Lactoperoxidase system for preservation of cosmetics and concluded that the Lactoperoxidase system can provide a broad-spectrum antimicrobial activity against bacteria, yeasts and moulds when it is composed of Lactoperoxidase, H_2O_2 and SCN at carefully selected weight ratios. Optimum results were obtained when H_2O_2 was generated enzymatically by the glucose oxidase-glucose system.

By his method cosmetics can be preserved for 2 to 4 months. A report by van Hooijdonk, Kussendrager, and Steijns (2000) indicated the potential of the lactoperoxidase system for use in fish farming, oral hygiene and functional foods. The LPsystem can also be used to eradicate L. monocytogenes from surfaces of fresh fish fillets and meat (Ramet, 2000).

Table1. Reports on the the development of enzyme immunoassays using peroxidase as a reporter enzyme to detect toxins , pathogens and other analytes (Carlos et al., 2004)

Pathiogen/analyte

product immunoaasy

Immuno assay

Goynyautoxins

shellfish tissue

Direct competative ELISA

T-2 toxin

maize, wheat, rye, barley

Flow-Through Enzyme Immunoassay

Ochratoxin A

Corn, barley, soybean

Direct competitive ELISA

Zearalenone toxins

Different cereals

Direct competitive ELISA

Fumonisin B1

Corn-based

Competitive EIA

Dengue virus proteins

Human serum

Immunoblots

Hepatitis-E virus

Human serum

ELISA

Alkaline

Milk Cotton seed

Competitive indirect

Streptomycin, dihydrostreptomycin

Milk

Double antibody solid phase EIA

Gossypol

Cotton seed

Non-competitive ELISA

Horseradish peroxidase in removal of phenol from waste water.

Phenolic compounds contaminate industrial waste water streams, mostly from the fibre industries, oil refineries and polymer processing industries. These are potential danger to human health because of toxicity and suspected to be carcinogenic (Sakurai et al., 2003). (Villalobos and

Buchanan, 2002), has reported conventional methods such as chemical, physical and biological process are not always suitable. Enzyme based treatment methods have several advantages because of their specificity, and are very efficient in removing targeted compounds and enzymes are easy to handle and store (Wilberg et al., 2002).

(Klibanov et al., 1983) The use of horse radish peroxidase (HRP) to eliminate phenol, was successfully demonstrated and proved enzymatic treatment was effective. In presence of enzyme peroxidase, phenols are oxidised to form the corresponding radicals through addition of hydrogen peroxide the radicals spontaneously react to rapidly form insoluble polymers, which can be easily precipitated from the waste water (A. Bodalo et al., 2005). In this case (HRP) is inactivated during enzymatic reaction due to interaction between the phenoxy radicals and/ or polymers produced to enzyme active site (A. Bodalo et al., 2005). The enzyme inactivation is minimized by addition of polyethylene glycol (PEG) it decreases the absorption of polymers onto the enzyme active site (A. Badalo et al.)

Production of peroxidase by hairy roots of Brassica napus:

The largest amount peroxidase is used in human medicine in clinical biochemical tests. Peroxidase production from (Brassica napus) is reliable source of commercial production of peroxidase. Agostini et al., 1996 produced peroxidase by hairy root cultures of turnip. Seeds of Brassica napus were surface sterilized in a 30% solution of domestic bleach for 2 mins . they were vacuum filtered and washed with three changes of sterile distilled water followed by soaking for 1 min in 70% ethanol . seeds were transferred to 50 % solution of domestic bleach containing 50 µl of tween 80

and were soaked for 15 to 20 min. Then they were washed three times with sterile distilled water and were placed on a MS Murashige and skoog medium containing 0. 9% w/v (oxoid). They were incubated for 30 days at 25 in dark /light regime of 16/8 hours under a light intensity of 15 µmoles m-2s-2.

Leaves of the resulting seedlings were used to produce hairy roots, obtained by inoculation of leaf explants with Agrobacterium rhizogenes strain. Infected explants were placed on solid MS medium containing vitamins and a 1 gm of ampicillian. Fifteen to eighteen days later adventitious roots appeared on the infected areas were excised and transferred to 125 ml flasks containing the same medium (without agar) for 25 to 30 days . terminal apices of the growing hairy roots were sub cultured to the same medium but without antibiotic and kept in orbital shaker at 100 rev/min and at 250c in the dark.

Complete hairy roots developed in the flask at each time sampling were vacuum filtered and dried between two sheets of filter paper . roots were homogenized in a mortar with 10mM acetic acetic buffer ph 4, containing 1 M nacl (1 g fresh weight per 10 ml buffer) at 40c . homogenates were centrifuged at 5000 g for 5mins and supernatants were used for enzyme assays.

Production of Peroxidase

The isolation of Peroxidase enzymes from field grown plants made difficult by the fact plants do not produce seeds and must therefore be propagated vegetatively (P. Soudek et al., 2005)

Seeds of Armorica rusticana L. were surface sterilized in 5%(m/v) sodium hypochlorite for 2min, soaked in sterile water three times for 10 mins, and germinated on hormone free Murashige and skoog (ms) medium at 27 0c, 16 photoperiod . after emergence of the second pair of leaves and nodel cuttings were transferred to fresh medium, and sub cultured at intervals of four weeks. Callus was cultured on hormone free MS medium at the same temperature and photoperiod.

Hair root cultures were obtained by inoculation of the callus cuttings with suspension of Agrobacterium rhizogenes. Hair root cultures were incubated on ms medium with o. 6 µm of naphthalene acetic acid (NAA) and 4. 44 um of -benzylaminopurine (BAP) and 500 mg ticarciline. Ticarciline was used for three subcultures cycles (2 weeks per cycle) to prevent growth of bacteria. In the second and third cultures cycle ms medium was either supplemented with o. 5 um

Four weeks old horse radish callus or tumors tissues grown in vitro were used as source for Peroxidase extraction. Complete hairy roots developed in the flask at each time sampling were vacuum filtered and dried between two sheets of filter paper . roots were homogenized in a mortar with 10mM acetic acetic buffer ph 4, containing 1 M nacl (1 g fresh weight per 10 ml buffer) at 40c . homogenates were centrifuged at 5000 g for 5mins and supernatants were used for commercial uses.

Conclusion

It was practically know that peroxidases have many possibilities for applications in clinically. Like In Diagnostic kits, antibody labellling, waste

water treatment and in food and paper industries. Peroxidase can be an efficient biocatalyst for the production of industrially relevant compounds. As peroxidase is produced by number of micro organisms and plants, plants source is used for commercial production.