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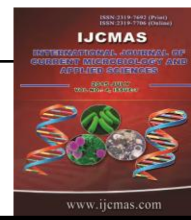

Review Article

A Review on Glucose Oxidase

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A B S T R A C T

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Glucose oxidase (GOX) from *Aspergillus niger* is a very much portrayed glycoprotein comprising of two indistinguishable 80-kDa subunits with two FAD co-factors bound. Both the DNA grouping and protein structure at 1.9 Ǻ have been resolved. GOX catalyzes the oxidation of D- glucose ($C_6H_{12}O_6$) to D-gluconolactone ($C_6H_{10}O_6$) and hydrogen peroxide. GOX production is natural in some fungi and insects where its reactant by-product, hydrogen peroxide, goes about as a hostile to bacterial and against fungal cultures. GOX is Generally Regarded as Safe (GRAS), and GOX from *A. niger* is the premise of numerous modern applications. GOX-catalyzed response uproots oxygen and produces hydrogen peroxide, a characteristic used in nourishment protection. This paper will give a brief foundation on the normal event, capacities, weaknesses of different chemicals that oxidize glucose, Isolation of and early deal with the protein, structure of GOD and how it identifies with the immobilization and strength of the chemical and in addition the properties of glucose oxidase.

Introduction

The living cell is the site of huge biochemical movement called metabolism. This is the procedure of concoction and physical change which goes on ceaselessly in the living organic entity. Fabricating up of new tissue, substitution of old one, change of nourishment to energy, transfer of waste materials, proliferation - all the exercises that we portray as "life."

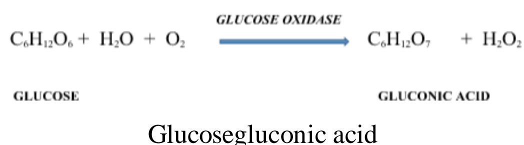
This building up and tearing down happens even with an evident oddity. The best lion's share of these biochemical reactions don't occur suddenly. The marvel of catalysis makes conceivable biochemical reactions fundamental for all life forms. Catalysis is characterized as the speeding up of a substance response by some substance which itself experiences no perpetual

concoction change. The impetuses of biochemical reactions are enzymes and are in charge of realizing the greater part of the synthetic reactions in living organic entities. Without enzymes, these reactions occur at a rate very moderate for the pace of metabolism.

Almost every known enzyme is protein. They are high molecular weight mixes made up chiefly of chains of amino acids connected together by peptide bonds.

In this paper we are concentrating on Glucose oxidase- industrially and biochemically essential catalyst. Just two non-hydrolytic enzymes at present have vast scale mechanical applications, glucose oxidase and catalase (Snyder, 1953).

Glucose oxidase is of fungal origin, and acts in the vicinity of oxygen to change over glucose to gluconic acid and hydrogen peroxide. It is profoundly particular and oxidizes just β-D-glucose.



Glucosegluconic acid

Catalase, which is also present in commercial fungal glucose oxidase preparations, acts on hydrogen peroxide to yield water and oxygen.

The net response of the glucose oxidase-catalase compound framework hence brings about one-half mole of oxygen being devoured for every mole of glucose oxidized.

The glucose oxidase-catalase framework is utilized industrially both for evacuating glucose and for uprooting oxygen. A fascinating application is likewise its utilization as a test reagent since it is particular for glucose. This proposal was first made by Keilin and Hartree (1948), and it has had extensive use in laboratories for this reason as a quantitative measure of glucose in the vicinity of different sugars (Whistler *et al*., 1953).

Business application is as paper test strips for diabetic patients, which show the vicinity of glucose in the urine by a shading change when the strip is plunged into the sample (Hunt *et al*., 1956). Various different uses for these test strips for subjective identification of glucose are additionally conceivable.

The enzymes that oxidize glucose

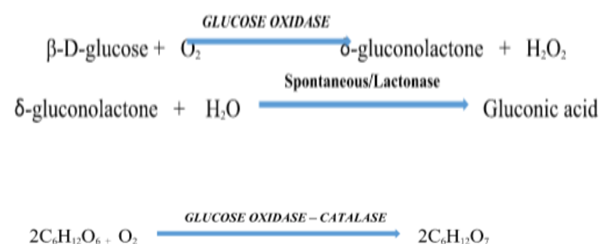
There are four sorts of enzyme that oxidize glucose as a key substrate:

Glucose dehydrogenases
Quinoprotein glucose dehydrogenases
Glucose I-oxidases
Glucose 2-oxidases

Glucose dehydrogenases (Metzger *et al.,* 1964, 1965) and quinoprotein glucose dehydrogenases (Hague, 1964, Duine *et al*., 1982) are both particular for β-D-glucose and have a high turnover. In any case, the previous obliges a soluble cofactor and the last is moderately temperamental. Glucose 2-oxidases (Janssen and Ruellius, 1968; Volt *et al*., 1985) oxidize glucose to glucosone. In the vicinity of dioxygen (o_2) the other item is hydrogen peroxide. Notwithstanding, they additionally oxidize different carbs like xylose and gluconolactone. This absence of specificity precludes the boundless utilization of this gathering of enzymes in biosensors for glucose, however they can be utilized to focus the aggregate sum of monosaccharides present in a sample (Olsson *et al.,* 1990).

The god mechanism

Glucose-1-oxidase (GOX) (β-D-glucose: oxygen-1- oxidoreductase, EC 1.1.3.4) is an all-around portrayed enzyme, which catalyzes the oxidation of β-D-glucose to D-gluconolactone and hydrogen peroxide (Wilson and Turner 1992). Both hydrogen peroxide and D-gluconolactone separates suddenly and synergistically. Notwithstanding this current, GOX's enzymatic action is lessened when hydrogen peroxide aggregates and inactivates the enzyme; the breakdown result of D-gluconolactone, gluconic acid ($C_6H_{12}O_7$) gathers, lessening pH of the arrangement. As anyone might expect, both gluconic acid (Miron *et al*., 2004) and hydrogen peroxide can bring about item hindrance of GOX.



Stability

Lyophilized GOD is amazingly steady. At 0°C it is stable for a long time such as 2 years and at -15°C for 8 years duration (Bentley, 1963). In solution stability is dependent on the pH. It is most steady at around pH 5. Beneath pH 2 or more pH 8 catalytic movement is quickly lost (Coulthard *et al*., 1945; Keilin and Hartree, 1948). At pH 8.1, for instance, just around 10% of the action stays after 10 min; at pH 9.1 inactivation is considerably more fast (Keilin and Hartree, 1948a). The rate of inactivation at high pH is decreased in the vicinity of glucose (Keilin and Hat-tree, 1948a). It is extremely impervious to proteolysis and is unaffected by delayed exposure to trypsin, pepsin andpapain (Coulthard*et al*., 1945; Keilin and Hartree, 19488).Non-ionic detergents have little impact on it, however movement is lost in the vicinity of ionic detergents like SDS and hexadecyltrimethylammonium bromide. Anionic detergents like SDS inactivate GOD at low pH (Jones *et al*., 1982) and cationic detergents like hexadecyl trimethylammonium bromide inactivate it at high pH. GOD has a generally low enthalpy of denaturation (450 kcal mol^{-1}) and is temperamental at temperatures in abundance of 40°C (Nakamura *et al*., 1976). To some degree it can be ensured against thermal denaturation by polyhydric alcohols like glycerol (Ye *et al*., 1988).

Inhibitors

GOD is restrained by micro-molar measures of substantial metals, for example, mercury, lead and silver (Nakamura and Ogura, 1962; Torren and Burger, 1968). The enzyme's defenselessness to these metals has been utilized to develop a biosensor intended to distinguish them (Liu *et al*., 1981). Millimolar measures of hydrazine, hydroxylamine and phenylhydrazine somewhat hinder the enzyme (Bentley, 1963). At a convergence of 10 mu the accompanying level of restraint is watched: 8- hydroxyquinoline (11%) sodium nitrate (13%) and semicarbazide (20%) (Keilin and Hartree, 1948). The movement of the enzyme additionally decreases in the vicinity of aldohexoses like D-arabinose (Adams *et al*., 1960) and 2-deoxy-D-glucose (Gibson *et al*., 1964) which go about as focused inhibitors. Halide particles repress GOD at low pH. At pH 3, for instance, it is totally restrained by 0.1 M potassium chloride. The capacity of polyamines to repress GOD (Voet and Andersen, 1984) has as of now been specified.

Physical characteristics

Glucose I-oxidase from *A. niger* is a somewhat stretched globular protein with an axial ratio of 2.5 : 1 (Nakamura *et al*., 1976), a normal breadth of 8 nm (Bourdillon *et al.*, 1980) and an incomplete particular volume of 0.75 ml g-1 (Swoboda andMassey, 1965). At 20°C the dissemination coefficient is 4.94 x 10-7 cm2 s-l in 0 1 M sodium chloride (Nakamura et al., 1976) and the sedimentation coefficient is 8.0 S at pH 5.5 (Swoboda andMassey, 1965; O'Malley and Weaver, 1972). Different qualities have been given for the sub-atomic mass in the reach 151× l03 to 186× l03 Da (Wellner, 1967) yet most values lie in the extent 155× l03 +5× l03 Da (O'Malley and Weaver, 1972). At 280 nm a 1% (w/v) solution of GOD has an absorbance of 16+ 0.32 cm-1 (Jones *et al.,* 1982). The absorbance spectrum has maxima at 278,382 and 452 nm, with absorbance values in the ratio 12.7: 0.92: 1.0(Swoboda and Massey, 1965). The termination coefficient at 450 nm is 1.41×104 (Swoboda andMassey, 1965). There is around 0.3 g of water connected with each gram dry weight of protein (Nakamura *et al*., 1976). The GOD from Penicillium amagasakiense has been acquired in an exceptionally immaculate solidified structure (Kusai *et al.*, 1960).

Gluconic acid production

Gluconic acid and its subsidiary salts are GRAS and can be utilized as a part of an extensive variety of businesses (Ramachandran *et al*. 2006) including material biting the dust, metal surface cleaning, nourishment added substances, cleansers, solid, beautifiers (Yu and Scott 1997) and pharmaceuticals (BACAS 2004). As a nourishment added substance, it can be utilized as an acidity controller, raising operators, shading stabilizer, cell reinforcement and chelating specialists in bread, sustain, drink, and so forth (Brookes et al. 2005; Codex Alimentarius Commission 2007a). Mechanically, gluconic acid is basically delivered from aging (Singh et al. 2005), with an expected worldwide generation of around 50,000–100,000 ton/year (BACAS 2004).

Similarly as with all aging procedures, there are a few weaknesses. Societies oblige different included supplements and no less than a couple of days to develop and perform bioconversion. Also, culture solutions create and contain undesirable by-items, require downstream refinements and devour substrates precluding high transformation proficiency. Consequently, the utilization of enzyme-based change is viewed as a suitable strategy to diminish generation cost and time (Nakao *et al.* 1997). For instance, amid 1997 and 2003, there were licenses recorded making cases of glucose-oxidase-based procedure that is equipped for right around 100% change proficiency, oblige less time than maturation and don't contain polluting influences (Vroemen and Beverini 1999; Lantero and Shetty 2004). Bioreactor utilizing immobilized glucose oxidase is one of the favored setups being explored (Godjevargova and Turmanova 2004).

Table.1 Properties of glucose oxidase from *A. niger*

Properties	Values
Molecular weight	150–186 kDa (Swoboda and Massey 1965; Nakamura and Fujiki 1968)
UV–VIS absorption (Tsuge *et al*., 1975)	280: 380: 450 (nm) 11.5: 1: 1.03 (ratio)
Extinction coefficient (M^{-1} cm^{-1})	270,000 (at 280 nm; Solomon *et al*., 1977) 25,180–28,200 (at 450 nm; Swoboda and Massey 1965; Johnson *et al*., 1989) 21,600 (at 452 nm; Nakamura and Fujiki 1968)
Specific activity (pH 5.6, 25–37°C)	80–172 µmol glucose/min/mg enzyme (Swoboda and Massey 1965)
Km (Michaelis constant, with respect to glucose)	198–248 mM (pH 5–7, 20–30°C, oxygen) 110–120 mM (pH 5.6, 0–38°C, oxygen; Gibson *et al*., 1964) 50–74 mM (pH 5.5, 15–30°C, oxygen; Nakamura and Ogura 1968b) 33 mM (pH 5.6, 25°C, oxygen; Swoboda and Massey 1965) 41.8 mM (pH 6.86, 25°C, benzoquinone)
Temperature range	20–50°C (Gouda *et al*., 2003)
pH range	4–7 (Keilin and Hartree 1947; Nakamura and Fujiki 1968)
Inhibitors	Ag+, Hg2+ and Cu2+ ions (µmol; Nakamura and Ogura 1968a;)Arsenite, p-chloromercuribenzoate, phenymercuric acetate (mmol; Nakamuraand Ogura 1968a) and others.
Isoelectric point (pI)	4.2 (Pazur and Kleppe 1964)

Fig.1 Glucose oxidase with its two subunits depicted as dark and light blue, while the FAD coenzyme is depicted as pink

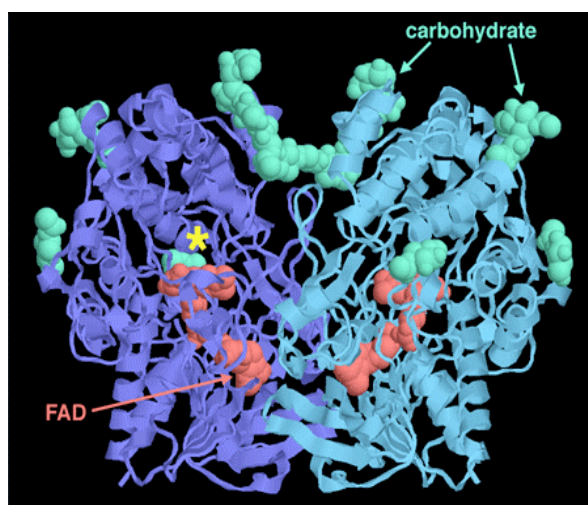
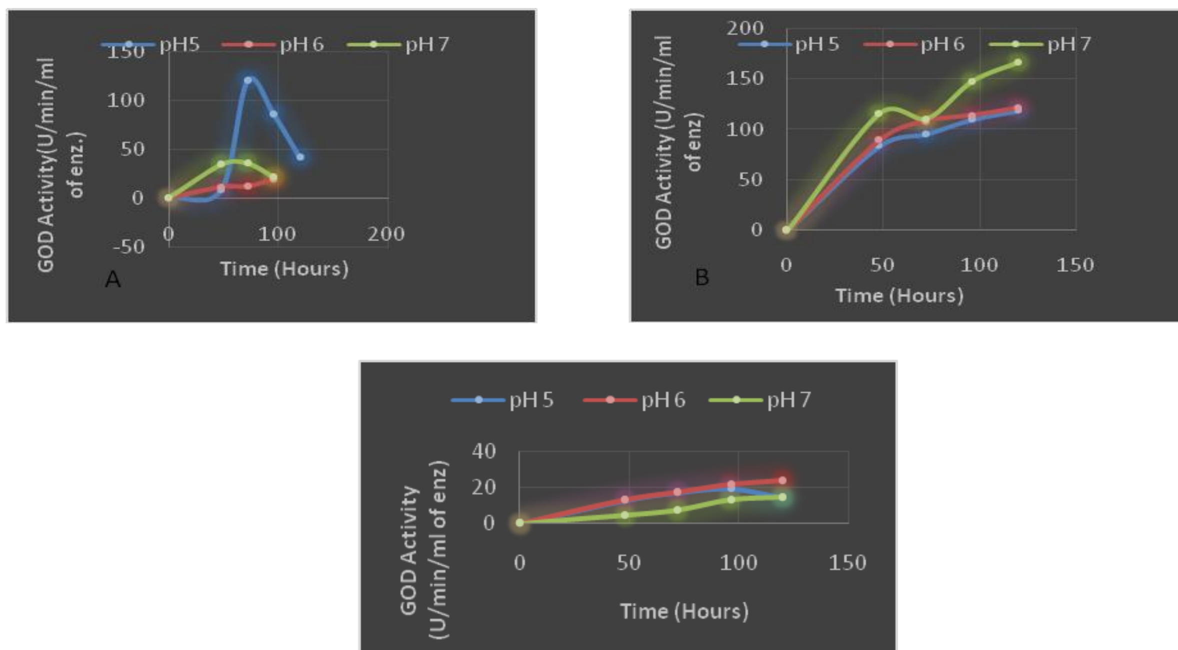


Fig.2 (A) showing the activity of GOD at initial 10% glucose (B) 10% sucrose (C) 10% lactose concentration and at 30^0 C on gyratory shaker. The more promising results can be obtained with sucrose, as carbohydrate source. [Shaikh, S.A. (2012) Immobilization and Production of gluconic acid under varying fermentation Conditions. (M.Phil. dissertation)]



Immobilization permits the enzymes to be reused, lessens cost and allowing moderately simpler outline and development of reactor to deliver and uproot of the wanted item, gluconic acid constantly. Regardless of these potential preferences, the absence of mechanical appropriation suggests that there are real obstacles to overcome before mass reception of enzyme-based bioconversion procedure happens.

The effect of pH on the mechanism

The majority of the work went for uncovering the activity of GOD has been completed at about pH 5.5 utilizing dioxygen as the electron acceptor. As said beforehand, the initial phase in the response of GOD with glucose is the development of an enzyme-substrate complex.

This is trailed by enzyme-base catalyzed lessening of FAD. At low pH protonation of the enzyme-base prompts an abatement in enzymatic action. At higher pH, GOD can be reoxidized. This, it is recommended, is less receptive towards dioxygen than the high-vitality structure and somewhat represents the diminishing in response rate seen at high pH.

Regardless of the impediments of this omnipresent enzyme, it's proceeded with utilization both in examination and down to earth applications is guaranteed. Further comprehension of its structure will incredibly upgrade the sub-atomic methodologies now being favored to accomplish the up and coming generation of bioelectronics gadgets like sugar sensors. Molecular biology and protein designing will have a vital part in enhancing the properties and proficiency of the enzyme for utilization in biosensors by, for instance, expanding soundness, decreasing the extent of the particle and enhancing the rate of

reactant and electron exchange forms. At last the part of biomimicry ought to be specified, for it might be that the most noteworthy commitment this enzyme makes to the fate of bioelectronics is as a model for future manufactured frameworks.

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