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Exploring the Intricacies and Functionalities of Galactose Oxidase: Structural Nuances, Catalytic Behaviors, and Prospects in Bio-electrocatalysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Galactose Oxidase, also known as GOase, is an enzyme found mostly in *Fusarium graminearum*, Dactylium dendroides, and *Gibberella fujikuroi*. GOase, containing copper, serves catalytic functions in oxidizing substrates and primary alcohols such as d-galactose, benzyl alcohol

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derivatives, and dihydroxyacetone. The catalytic property of galactose oxidase that differentiates it from other enzymes is its cofactor consisting of a Cu (II)-bound Cys-Tyr* radical. The cofactor is vital for enabling regioselective oxidation. The application of galactose oxidase (GOase) covers several fields such as enzymatic synthesis, biosensors development, and processes of diagnosis.

Galactose oxidase (GOase) was discovered to have a crystallographic structure by X-ray diffraction, which revealed an active site containing copper ions displaying relatively square pyramidal geometry. There are three unique structural and functional domains of the enzyme GOase. The domains include Tyr495 and a covalent bond between Cys228 and Tyr272 serving as equatorial and axial ligands, respectively. The mechanism of catalysis covers three different oxidation states, which include the active state containing Cu (II)-radical, the intermediate state containing Cu (II)-tyrosine, and the Cu(I)-tyrosine state. The cycle of catalysis that has been posited comprises several phases which are: (i) substrate binding, (ii) the transfer of proton (iii) the transfer of hydrogen atom, and (iv) subsequent oxidation steps. These phases eventually yield the synthesis of aldehyde and hydrogen peroxide.

Galactose oxidase's (GOase) mechanism of catalysis has been studied thoroughly via extensive research focusing on explaining the ping-pong mechanism that occurs in both oxidative and reductive half-reactions. The processes of activating and reactivating galactose (GOase) involve the transfer of electrons in which horseradish peroxidase (HRP) serves as an activator. The electrochemical investigations provide evidence of the electrochemical activation and reactivation of GOase in the presence of mediators.

This comprehensive review enhances the comprehension of the structural complexities, catalytic mechanisms, and bio-electrocatalytic potential of GOase, thereby establishing a basis for future investigations and developments in technology.

Keywords: Galactose oxidase; goase; catalytic property; enzymatic synthesis; biosensors development; processes; aldehyde; hydrogen peroxide; catalysis; glycoproteins; biocatalytic conversion.

1. INTRODUCTION

In biochemical catalysis, free radicals have become one of the fundamental characteristics [1-3] in relation to enzymes that have developed techniques to exploit radical chemistry in the activation of bonds and rearranging molecules. Free radicals' rare chemical reactivity is traceable to the unpaired electrons present in their electronic valence shell, which makes them somewhat transition metal ions' organic analog [4]. Radicals are recognized to play vital biological functions, and while, in history, the focus was initially on their deleterious impacts, enormous evidence currently exists that radicals take part in several key life processes such as photosynthesis, DNA replication, and respiration [5].

Free radicals are known to be essential components in a wide range of enzymatic mechanisms such as aminomutase [6], biotin synthase [7], cytochrome c peroxidase [8], lipoyl synthase [9], ribonucleotide reductase [10], pyruvate-formate lyase [11], prostaglandin H synthase [12], diol dehydrase [13], and DNA photolyase [14] amidst others. Galactose oxidase [15], the enzyme secreted by fungi, used broadly in histological and bioanalytical applications, is among the most characterized of the enzymes carrying free radicals.

Overall, the galactose oxidase-catalyzed reaction is primary alcohol oxidation to the equivalent aldehyde, bound to dioxygen reduction to hydrogen peroxide [16].

The product which is of biological importance:

$$RCH_2OH + O_2 \rightarrow RCHO + H_2O_2$$

The reaction catalysis is theoretically equal to a dihydrogen elements' transfer between both substrates as Oxygen reduction and alcohol oxidation are two-electron processes. The transfer of hydrogen biologically typically entails specialized organic redox cofactors (for instance, nicotinamide, flavins, and quinones), having reaction mechanisms that are well-characterized. Galactose oxidase lacks these redox cofactors and employs a much different active site – a copper complex bound to a free radical – to carry out this chemistry [17]. This paper aims to assess the chemistry of the enzyme Galactose Oxidase to better understand its structure, mechanism of action, and overall application of the enzyme.

2. OVERVIEW OF GALACTOSE OXIDASE

Galactose oxidase (E.C.1.1.3.9) also termed GOase is a metalloenzyme of copper contained primarily in three fungi – *Fusarium graminearum, Gibberella fujikuroi* and *Dactylium dendroides* (the widely characterized) [18]. GOase is classed under the type II mononuclear copper-containing enzyme and is made up of one single polypeptide (molecular mass: 68kDa) [19]. GOase is involved in catalyzing disomers' oxidation of a wide primary alcohols' variety – dihydroxyacetone (DHA), d-galactose [20], and benzyl alcohols' substitutes [21], to their equivalent aldehyde, linked with dioxygen's reduction to hydrogen peroxide [22].

GOase can be applied in a wide range of areas such as diagnCu (ics, enzymatic synthesis, and biosensors [23,24]. A germane characteristic of GOase in catalysis is the rare Cu (II)-bound Cys-Tyr• radical cofactor contained in its active site via its free radical tyrosyl that coordinates directly to the type II copper center [25]. The primary alcohols' regioselective oxidation, comprising galactose and others that range from allyl alcohol, and glycerol to oligo- and polysaccharides, galactopyranosides is performed by the Cu (II)-radical cofactor [26]. A dioxygen $2e^-$ oxidation oxidizes the reduced Cu(I)-(Tyr-Cys) to an active Cu (II)-(Tyr•-Cys) form (Fig. 1).

3. STRUCTURE OF GALACTOSE OXIDASE

In 1991, The GOase crystallographic structure was determined successfully using X-ray diffraction at a 1.7 A resolution. It showed a fascinating characteristic of the copper ion active site that an almost square pyramidal geometry coordinates [27]. The structure of the enzyme was explicated totally; three diverse domains, primarily composed of short turns *β*-structure, were differentiated functionally and structurally (Fig. 2a). As an axial ligand, Tyr495 coordinates the active site's copper, while as an equatorial ligand, there is Tyr272, His486, His581, and a water (solvent) molecule (pH 7) with weak coordination. Also, the scholars inferred that a second organic cofactor exists that is derived from the covalent linkage between Tyr272 and Cys228 post-translationally. The protein reactiveness and structure are affected by the thioether bond linking the two residues [28]. Substantial interests have been drawn by this characteristic, leading to comprehensive spectroscopic research [29], primarily the resonance of electron paramagnet (5**),** crystallography of X-ray (6,7), and site-directed mutagenesis study [30-31], which haveresulted in a rational understanding of the mechanism of catalysis. The GOase active site's Tyr272 a

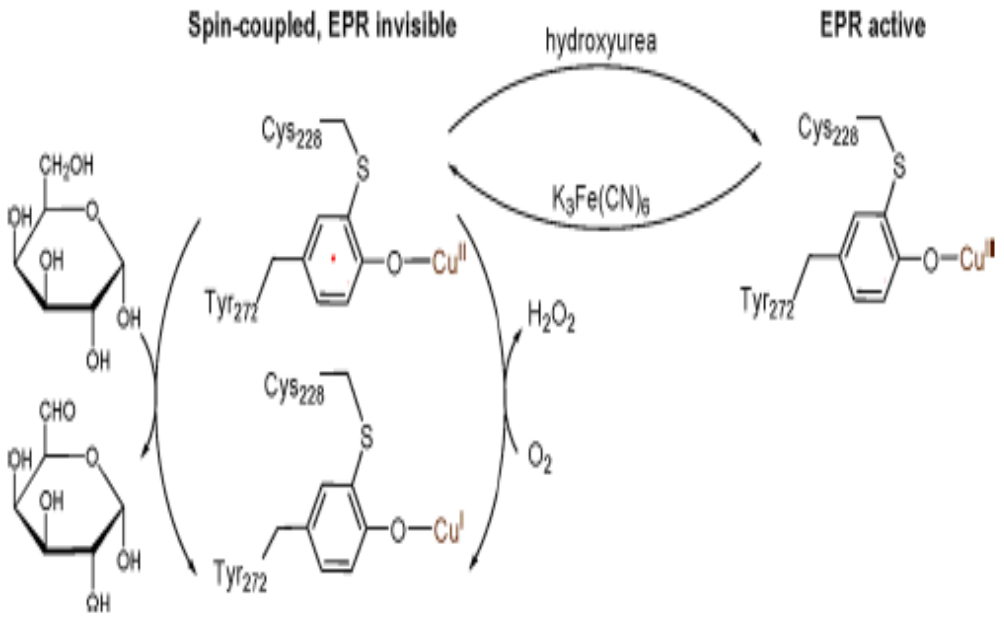

Fig. 1. GOase catalytic reaction and three cu (ii)-(tyr•-cys) center the oxidation states

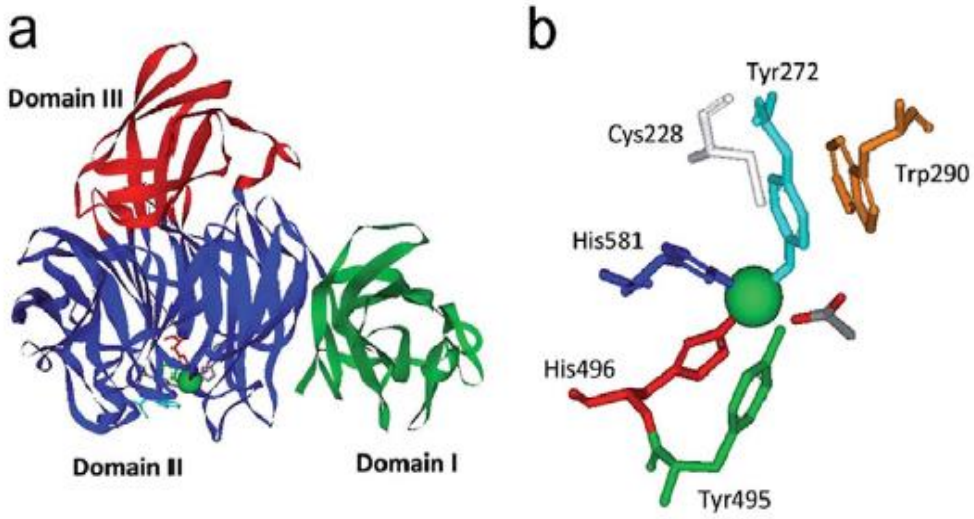

Fig. 2. Dactylium dendroides' galactose oxidase structures. (a) Enzyme's structural illustration (in 3-D) with the three domains shown. (b) The explicated catalytic active site's structure. Source: (Ikemoto et al., 2015)

cooper can be in three unique oxidation states as follows: (1) the catalytically active state having Cu (II) and tyrosyl radical (GOaseox), the Cu (II) and tyrosine intermediate state (GOasesemi), and the last state having Cu(I) and tyrosine (GOasered) [32]. Whittaker's posited mechanism of catalysis regards the initial step as the binding, to the equatorial cooper site, of the substrate, removing the water ligand, following a proton transfer from the alcohol to the axial Tyr495. Then, in an explicated step from the experiments' substitution of isotope, a hydrogen atom is transferred to the tyrosyl radical from the substrate. The substrate's produced ketyl radical is thereafter oxidized via the transfer of electrons to the cooper center, producing Cu(I) and aldehyde. Lastly, Cu(I) and tyrosine are re-oxidized through oxygen molecules, re-yielding Cu (II) and tyrosyl while hydrogen peroxide is produced as a sub-product [33].

4. CATALYTIC MECHANISM OF GOase

As stated earlier, there are three unique oxidation states of GOase: the oxidized state having Cu (II) and tyrosyl radical, the Cu (II) and tyrosine intermediate (semi-reduced) state, and the reduced state having Cu(I) and tyrosine. The GOase-catalyzed reaction begins with substrate bound before the Cu ion and can be classified as an oxidative and reductive half-reaction via a ping-pong mechanism [34]. Based on the first catalytic mechanism posited, the oxidized free-radical, that is, catalytically active Cu (II)-complex (of tyrosyl radical and Cu (II)) is reduced, in the reductive half-reaction, to the non-radical Cu(I)-complex (of tyrosine and Cu(I)) in three steps (Fig. 3).

Firstly, there is a proton transfer, to the tyrosinate (Tyr 495), from the alcohol (substrate). The axial Tyr 495 was posited to function as a common base for the extraction of the proton from the matched hydroxyl group and was evidenced to be vital for catalysis [35]. Although containing both the cysteine-tyrosyl radical co-factor and copper, the mutant of Y495F is inactive. Thereafter, in the following step, there is a transfer of hydrogen atoms, to the tyrosyl radical (Tyr272), from the substrate. The step is regarded as at least partially or entirely rate-limiting based on spectroscopic research [36] The alkoxyl radical derived from alcohol that remains is oxidized by the transfer of electrons to the cooper ion-producing the product aldehyde and Cu(I). Based on research using inhibitors, electron and hydrogen atom transfer was posited to ensue in a concerted way [37]. The latest research theories showed that before the first proton transfer, the location of the radical site was at the axial Tyr495. Alongside the transfer of the proton, the radical is moved from the Cys-Tyr-dimer to the Tyr272. Hence, an assumption was made that the transfer of an electron to Cu(I)

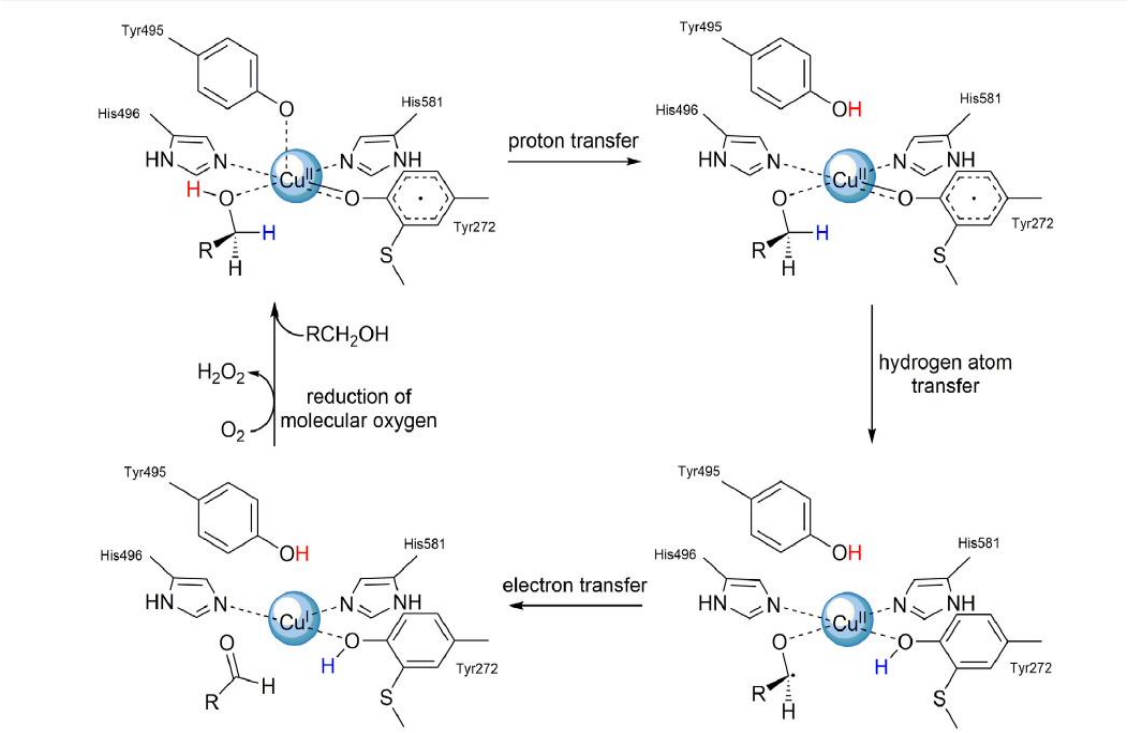

Fig. 3. Posited GOase cycle of catalysis from *F. graminearum*

from the alkoxyl radical intermediate cannot be extremely exothermic, as in the reaction, the rate-limiting step would be that involving O_2 reduction [38].In the oxidative half-reaction that follows, when the release of the aldehyde (product) from the active site has occurred, whereas molecular oxygen occupies its position in front of the cooper ion, the molecular oxygen oxidizes the reduced non-radical Cu(I)-complex producing hydrogen peroxide and Cu (II)-complex free-radical having tyrosyl radical and Cu(II) (Fig. 3) [39].An electron was projected to be transferred from Cu(I) to the O_2 bound, whereas O_2 receives a hydrogen atom by transfer from Tyr272 (absent in Fig. 3). Following the transfer of a proton from the TYR495 phenol H_2O_2 exits the reaction [40]. The oxidized Cu (II)-complex free-radical is somewhat stable but can be simply reduced, through an electron transfer, to the catalytically inactive non-radical Cu (II)-complex by the action of a wide chemicals' spectrum [41]. The next single-electron reduction of the complex results in the non-radical Cu(I)-complex formation that can interact with O_2 and therefore re-introduces GOase into the cycle of reactions. To prevent inactive GOase accumulation in the reaction, which lowers the rate of reaction and yields partial conversion of the substrate, potassium ferricyanide (a mild oxidant) can be introduced into the reaction for GOase reactivation [42]. The oxidized Cu (II)-complex free-radicals redox potential was discovered to be modulated by Trp290 amassing to the Cys-Tyr-dimer. Although GOase wild-type via potassium ferricyanide ($E^{\circ\prime}$= 424 mV) was oxidized to the Cu (II)-complex free-radical, treatment with cesium octacyanomolybdate ($E^{\circ\prime}$= 892 mV) was required to oxidize the W290H variant [43]. GOase was discovered to be activated by Horseradish peroxidase (HRP) [44-45]. HRP was posited to be an H_2O_2 scavenger, from the reaction GOase catalyzes, hence, the enzyme is protected from deactivation [46]. Subsequently, HRP's function was reassessed, and a postulation was given that it serves as a GOase activator, [47]. There is no complete understanding of the activating effect but was described using its role as a one-electron oxidant needed to regenerate, upon decay, the radical of the active site [48]. Therefore, commonly, HRP is included, during reactions, to enhance GOase activity. Usually, catalase is introduced for the decomposition of deleterious H_2O_2 to water and O_2 as H_2O_2 is a GOase inactivator and inhibitor, therefore oxygen is reintroduced into the reaction and the entire requirement for O_2 is reduced [49-51]. Lately, through cyclic voltammetry, studies have been conducted on the GOase electrochemical activation from *F. graminearum* and its developed variant in the presence of

many mediators at pH 7-9 [52].The dependence of the rates of electron transfer on both the pH value and the redox potential of the mediator has been demonstrated. GOase oxidation at pH values 7-9 by mediators was posited to follow a concentrated proton-couples electron transfer (PCET) mechanism in anaerobic settings. Furthermore, GOase variants mediated electrochemical re-activation was applied while oxidizing diverse alcohols. Both GOase HRP-mediated and electrochemical activation yielded the same conversion values of substrate and yields of product. Although the conversion and selectiveness had sensitivity to the operational voltage, there was no observed correlation between the redox potential and the conversion of the studied mediators.

5. STUDIES ON THE DIRECT ELECTRON TRANSFER OF GOASE

In the attempt to make the natural activity of redox enzymes be coupled to an electrode, there is usually a huge issue in accomplishing the direct electron transfer (DET) between the active site of the enzyme and the surface of the electrode. Significantly effective DET with electrodes has been demonstrated in metalloenzymes possessing redox centers near the surface of the protein, for example, multi-cooper oxidases and hydrogenases [53-54]. However, GOase DET with electrodes is somewhat elusive even though it has the site of its cooper redox close to the surface of the protein (about 8 Å). Few reports exist in literation in comparison to other proteins that contain coopers, like bilirubin oxidases, laccases, or azurin [55].About 20 years ago, Tkac et al. asserted that there is indirect proof of the GOase direct electron transfer to graphite electrodes by detecting enzyme activation on the application of redox potential over 150 mV versus SCE, concurring roughly with the one on its active site's tyrosine radical (Table 1), was recognized [56].On covering the surface of the electrode with a membrane of cellulose acetate to separate it from GOase, the effect of activation by the application of redox potential then disappeared. Although in this electrochemical research, the graphite surface was modified, prior, with an adsorbed ferrocene, hence, GOase-mediated electron transfer cannot be eliminated in such circumstances. GOase DET has been examined by the deposition of GOase on gold electrodes with diverse thiols' self-assembled monolayers (SAM) [57] When short-chain hydrophilic thiols are involved, because of the adsorbed GOase, sharp peaks were seen using cyclic voltammetry (CV). Notwithstanding, there were highly unstable redox signals and lacked bio-electrocatalytic impact on the introduction of galactose which can be linked with them, signifying that they had correspondence with the denatured enzyme.

GOase entrapment in a compound film of poly (Lactide-capped Au nanoparticles and reduced graphene oxide applied on glass-like carbon electrodes have been examined for an indication of DET. There was an identified symmetrical quasi-reversible redox process using cyclic voltammetry having a formal potential of -137mV versus SCE. The value of this potation is relatively low in comparison to those projected for the GOase active site's redox centers (Table 1). Additionally, no currents of oxidative electrocatalysis could be linked with the identified redox process, on the addition of galactose, using CV as indicated. Rather, more currents of reduction in the presence of galactose were assessed by chronoamperometry at -0.42V versus SCE in an oxygen-saturated buffer [58]. There was no explanation regarding the unanticipated outcome, as GOase uses up

Table 1. Redox potential of electrodes

Electrode	Technique	Redox Potential (mV versus NHE)	References
Au and GOase in solution	Spectro electrochemical titration	+410 (Tyr·/Tyr) and +159 ($Cu^{2+/1+}$)	59
Au/SAM/GOase	DPV	+440 to +460 and +130 to +200	60
Au/SAM/AuNP-GOase		+440 and +265	61
GCE/RGO/AuNP/GOase	CV	+107	62
Au/SAM/CNT/GOase	CV	+350 to +390	63
FTO/TIO_2/GOase	CV	+300	64

oxygen during its cycle of catalysis [65-67]. Consequently, the cathodic current as a result of direct oxygen reduction at the electrode should reduce galactose addition if the static GOase is active [68-70].

6. CONCLUSION

Galactose Oxidase is a cooper-containing enzyme found in three major fungi organisms - *Fusarium graminearum, Gibberella fujikuroi,* and *Dactylium dendroides* and has been associated with the catalysis of the oxidation reaction of primary alcohols, and other substrates such as allyl alcohols as well as oligo- and polysaccharides. It has applications in several areas such as biosensor, diagnostics, and other fields. Furthermore, its mechanism of catalysis involves several steps in a catalytic cycle with a rate-limiting reaction that takes part in the oxidation of substrates. Several studies have also been conducted to reveal the direct electron transfer (DET) that takes place in the enzyme during catalysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Van Stappen C, Deng Y, Liu Y, Heidari H, Wang JX, Zhou Y, Lu Y. Designing artificial metalloenzymes by tuning of the environment beyond the primary coordination sphere. Chemical Reviews. 2022;122(14):11974-12045.
2. Guengerich FP. Mechanisms of cytochrome P450-catalyzed oxidations. ACS catalysis. 2018;8(12); 10964-10976.
3. Houée-Lévin C, Bobrowski K, Horakova L, Karademir B, Schöneich C, Davies MJ, Spickett CM. Exploring oxidative modifications of tyrosine: an update on mechanisms of formation, advances in analysis and biological conse-quences. Free Radical Research. 2015;49(4): 347-373.
4. Wang R, Rebek J, Yu Y. Organic radical reactions confined to containers in supramolecular systems. Chemical Communications. 2022;*58*(12):1828-1833.
5. Radi R. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. Proceedings of the National Academy of Sciences. 2018;115(23):5839-5848.
6. Kyne SH, Lefèvre G, Ollivier C, Petit M, Cladera VAR, Fensterbank L. Iron and cobalt catalysis: New perspectives in synthetic radical chemistry. Chemical Society Reviews. 2020;49(23):85018542.
7. Bockman MR, Engelhart CA, Cramer JD, Howe MD, Mishra NK, Zimmerman M, Aldrich CC. Investigation of (S)-(−)-acidomycin: A selective antimycobacterial natural product that inhibits biotin synthase. ACS Infectious Diseases. 2019; *5*(4):598-617.
8. Yoshikawa S, Shimada A. Reaction mechanism of cytochrome c oxidase. Chemical Reviews. 2015;115(4): 1936-1989.
9. Dong G, Cao L, Ryde U. Insight into the reaction mechanism of lipoyl synthase: A QM/MM study. JBIC Journal of Biological Inorganic Chemistry. 2018;23: 221-229.
10. Aye Y, Li M, Long MJC, Weiss RS. Ribonucleotide reductase and cancer: Biological mechanisms and targeted therapies. Oncogene. 2015;34(16):2011-2021.
11. Cáceres JC, Dolmatch A, Greene BL. The mechanism of inhibition of pyruvate formate lyase by methacrylate. Journal of the American Chemical Society. 2023; 145(41):22504-22515.
12. Tsai AL, Kulmacz RJ. Prostaglandin H synthase: Resolved and unresolved mechanistic issues. Archives of Biochemistry and Biophysics. 2010;493 (1):103-124.
13. Bilić L, Barić D, Sandala GM, Smith DM, Kovačević B. Glycerol as a substrate and inactivator of coenzyme B12-Dependent diol dehydratase. Chemistry–A European Journal. 2021;*27*(29):7930-7941.
14. Zhang M, Wang L, Zhong D. Photolyase: Dynamics and electron-transfer mechanisms of DNA repair. Archives of Biochemistry and Biophysics. 2017;632: 158-174.
15. Figueiredo C, De Lacey AL, Pita M. Electrochemical studies of galactose oxidase. Electrochemical Science Advances. 2022;*2*(5):e2100171.
16. Mathieu Y, Cleveland ME, Brumer H. Active-site engineering switches carbohydrate regiospecificity in a fungal copper radical oxidase. *ACS* Catalysis, 2022;12(16):10264-10275.

17. Quist DA, Diaz DE, Liu JJ, Karlin KD. Activation of dioxygen by copper metalloproteins and insights from model complexes. JBIC Journal of Biological Inorganic Chemistry. 2017;*22*:253288.
18. Whittaker MM, Whittaker JW. The active site of galactose oxidase. Journal of Biological Chemistry. 1988;263(13):6074-6080.
19. Paukner R, Staudigl P, Choosri W, Haltrich D, Leitner C. Expression, purification, and characterization of galactose oxidase of Fusarium sambucinum in E. coli. Protein Expression and Purification. 2015;108: 73-79.
20. Kanyong P, Krampa FD, Aniweh Y, Awandare GA. Enzyme-based amperometric galactose biosensors: A review. Microchimica Acta. 2017;184: 3663-3671.
21. Whittaker MM, Whittaker JW. Catalytic reaction profile for alcohol oxidation by galactose oxidase. Biochemistry. 2001;40 (24):7140-7148.
22. Ikemoto H, Mossin SL, Ulstrup J, Chi Q. Probing structural and catalytic characteristics of galactose oxidase confined in nanoscale chemical environments. RSC Advances. 2014;4(42): 21939-21950.
23. Kanyong P, Krampa FD, Aniweh Y, Awandare GA. Enzyme-based amperometric galactose biosensors: A review. Microchimica Acta. 2017;184:3663-3671.
24. Nie Y, Liu Y, Zhang Q, Su X, Ma Q. Novel coreactant modifier-based amplified electrochemiluminescence sensing method for point-of-care diagnostics of galactose. Biosensors and Bioelectronics. 2019; 138:111318.
25. Ito N, Phillips SE, Yadav KD, Knowles PF. Crystal structure of a free radical enzyme, galactose oxidase. Journal of Molecular Biology. 1994;*238*(5):794-814.
26. Avigad G. Oxidation rates of some desialylated glycoproteins by galactose oxidase. Archives *of* Biochemistry and biophysics. 1985;*239*(2):531-537.
27. Ito N, Phillips SE, Stevens C, Ogel ZB, McPherson MJ, Keen JN, Knowles PF. Novel thioether bond revealed by a 1.7 Å crystal structure of galactose oxidase. Nature. 1991;350(6313):87-90.
28. Whittaker JW. Galactose oxidase. Advances in Protein Chemistry. 2002;60: 1-49.
29. Deacon SE, Mahmoud K, Spooner RK, Firbank SJ, Knowles PF, Phillips SE, McPherson MJ. Enhanced fructose oxidase activity in a galactose oxidase variant. Chem Bio Chem. 2004;5(7):972-979.
30. Reynolds MP, Baron AJ, Wilmot CM, Vinecombe E, Stevens C, Phillips SE, McPherson MJ. Structure and mechanism of galactose oxidase: Catalytic role of tyrosine 495. JBIC Journal of Biological Inorganic Chemistry. 1997;*2*: 327-335.
31. Saysell CG, Barna T, Borman CD, Baron AJ, McPherson MJ, Sykes AG. Properties of the Trp290His variant of Fusarium NRRL 2903 galactose oxidase: interactions of the GOase semi state with different buffers, its redox activity and ability to bind azide. JBIC Journal of Biological Inorganic Chemistry. 1997;2: 702-709.
32. Himo F, Eriksson LA., Maseras F, Siegbahn PE. Catalytic mechanism of galactose oxidase: A theoretical study. Journal of the American Chemical Society. 2000;122(33):8031-8036.
33. Whittaker MM, Whittaker JW. Ligand interactions with galactose oxidase: Mechanistic insights. Biophysical Journal. 1993;64(3):762-772.
34. Whittaker JW. The radical chemistry of galactose oxidase. Archives of Biochemistry and Biophysics. 2005;433 (1):227-239.
35. Whittaker JW. Galactose oxidase. Advances in Protein Chemistry. 2002;60:1-49.
36. Whittaker MM, Whittaker JW. Ligand interactions with galactose oxidase: Mechanistic insights. Biophysical Journal. 1993;64(3):762-772.
37. Wachter RM, Branchaud BP.). Thiols as mechanistic probes for catalysis by the free radical enzyme galactose oxidase. Biochemistry. 1996;*35*(45):14425-14435.
38. Himo F, Eriksson LA., Maseras F, Siegbahn PE. Catalytic mechanism of galactose oxidase: A theoretical study. Journal of the American Chemical Society. 2000;122(33):8031-8036.
39. Whittaker JW. The radical chemistry of galactose oxidase. Archives of Biochemistry and Biophysics. 2005;433(1): 227-239.

40. Whittaker JW. Oxygen reactions of copper oxidases. Essays in Biochemistry. 1999; 34:155-172.
41. Whittaker MM, Whittaker JW. The active site of galactose oxidase. Journal of Biological Chemistry. 1988;263(13):6074-6080.
42. Whittaker JW. Free radical catalysis by galactose oxidase. Chemical Reviews. 2003;103(6):2347-2364.
43. Rogers MS, Tyler EM, Akyumani N, Kurtis CR, Spooner RK, Deacon SE, Dooley DM. The stacking tryptophan of galactose oxidase: A second-coordination sphere residue that has profound effects on tyrosyl radical behavior and enzyme catalysis. Biochemistry. 2007;46(15):4606-4618.
44. Kwiatkowski LD, Kosman DJ. On the role of superoxide radical in the mechanism of action of galactose oxidase. Biochemical and Biophysical Research Communications. 1973;53(3):715721.
45. Tressel P, Kosman DJ. o, o-Dityrosine in native and horseradish peroxidase-activated galactose oxidase. Biochemical and Biophysical Research Communications. 1980;92(3):781-786.
46. Kwiatkowski LD, Kosman DJ. On the role of superoxide radical in the mechanism of action of galactose oxidase. Biochemical and Biophysical Research Communications. 1973). 53(3), 715-721.
47. Tressel P, Kosman DJ. o, o-Dityrosine in native and horseradish peroxidase-activated galactose oxidase. Biochemical and Biophysical Research Communications. 1980;92(3):781786.
48. Toftgaard Pedersen A, Birmingham WR, Rehn G, Charnock SJ, Turner NJ, Woodley JM. Process requirements of galactose oxidase catalyzed oxidation of alcohols. Organic Process Research & Development. 2015;19(11):1580-1589.
49. Birmingham WR, Toftgaard Pedersen A, Dias Gomes M, Bøje Madsen M, Breuer M, Woodley JM, Turner NJ. Toward scalable biocatalytic conversion of 5-hydroxymethylfurfural by galactose oxidase using coordinated reaction and enzyme engineering. Nature Communications. 2021;12(1):4946.
50. Forget SM, Xia FR, Hein JE, Brumer H. Determination of biocatalytic parameters of a copper radical oxidase using real-time reaction progress monitoring. Organic & Biomolecular Chemistry.2020;18(11): 2076-2084.
51. Toftgaard Pedersen A, Birmingham WR, Rehn G, Charnock SJ, Turner NJ, Woodley JM. Process requirements of galactose oxidase catalyzed oxidation of alcohols. Organic Process Research & Development. 2015;19(11):1580-1589.
52. Zhang S, Ruccolo S, Fryszkowska A, Klapars A, Marshall N, Strotman NA. Electrochemical activation of galactose oxidase: Mechanistic studies and synthetic applications. ACSCatalysis. 2021;11(12): 7270-7280.
53. Vincent KA, Parkin A, Armstrong FA. Investigating and exploiting the electrocatalytic properties of hydrogenases. Chemical Reviews. 2007; 107(10):4366-4413.
54. Shleev S, Tkac J, Christenson A, Ruzgas T, Yaropolov AI, Whittaker JW, Gorton L. Direct electron transfer between copper-containing proteins and electrodes. Biosensors and Bioelectronics. 2005;20 (12):2517-2554.
55. Shleev S, Tkac J, Christenson A, Ruzgas T, Yaropolov AI, Whittaker JW, Gorton L. Direct electron transfer between copper-containing proteins and electrodes. Biosensors and Bioelectronics. 2005; 20(12):2517-2554.
56. Tkac J, Vostiar I, Gemeiner P, Sturdik E. Indirect evidence of direct electron communication between the active site of galactose oxidase and a graphite electrode. Bioelectrochemistry. 2002;*56*(1-2):23-25.
57. Shleev S, Tkac J, Christenson A, Ruzgas T, Yaropolov AI, Whittaker JW, Gorton L. Direct electron transfer between copper-containing proteins and electrodes. Biosensors and Bioelectronics. 2005;20 (12):2517-2554.
58. Xie J, Chen C, Zhou Y, Fei J, Ding Y, Zhao, J. A galactose oxidase biosensor based on graphene composite film for the determination of galactose and dihydroxyacetone. Electroanalysis. 2016; 28(1):183-188.
59. Shleev S, Tkac J, Christenson A, Ruzgas T, Yaropolov AI, Whittaker JW, Gorton L. Direct electron transfer between copper-containing proteins and electrodes. Biosensors and Bioelectronics. 2005;20 (12):2517-2554.
60. Shleev S, Tkac J, Christenson A, Ruzgas T, Yaropolov AI, Whittaker JW, Gorton L. Direct electron transfer between copper-containing proteins and electrodes.

Biosensors and Bioelectronics. 2005;20 (12):2517-2554.
61. Abad JM, Gass M, Bleloch A, Schiffrin DJ. Direct electron transfer to a metalloenzyme redox center coordinated to a monolayer-protected cluster. Journal of the American Chemical Society. 2009;131(29):10229-10236.
62. Xie J, Chen C, Zhou Y, Fei J, Ding Y, Zhao J. A galactose oxidase biosensor based on graphene composite film for the determination of galactose and dihydroxyacetone. Electroanalysis. 2016;28(1):183-188.
63. Wayu MB, Pannell MJ, Labban N, Case WS, Pollock JA, Leopold MC. Functionalized carbon nanotube adsorption interfaces for electron transfer studies of galactose oxidase. Bioelectrochemistry. 2019;125:116-126.
64. Qu J, Wang P, Wang Y, Li Z, Yang F, Han C, Yu D. Determination of phospholipids in soybean oil using a phospholipase-choline oxidase biosensor based on g-C3N4-TiO2 nanocomposite material. Journal of Food Composition and Analysis. 2023;124: 105717.
65. Olaniyi OO, Asonze CU, Ajayi SA, Olabanji, SO. Adigwe CS. A Regressional study on the impact of organizational security culture and transformational leadership on social engineering awareness among bank employees: The interplay of security education and behavioral change. Asian Journal of Economics, Business and Accounting. 2023;23(23):128–143.
Available:https://doi.org/10.9734/ajeba/2023/v23i231176
66. Olaniyi OO, Shah NH, Bahuguna N.). Quantitative analysis and comparative review of dividend policy dynamics within the banking sector: insights from global and U.S. Financial data and existing literature. Asian Journal of Economics, Business and Accounting. 2023;23(23): 179–199.
Available:https://doi.org/10.9734/ajeba/2023/v23i231180
67. Olaniyi OO, Omubo DS. The importance of COSO framework compliance in information technology auditing and enterprise resource management. The International Journal of Innovative Research & Development; 2023.
Available:https://doi.org/10.24940/ijird/2023/v12/i5/MAY23001
68. Olaniyi OO, Omubo DS. WhatsApp data policy, data security, and users' vulnerability. The International Journal of Innovative Research & Development; 2023.
Available:https://doi.org/10.24940/ijird/2023/v12/i4/APR23021
69. Omogoroye OO, Olaniyi OO, Adebiyi OO, Oladoyinbo TO, Olaniyi FG. Electricity consumption (kW) forecast for a building of interest based on a time series nonlinear regression model. Asian Journal of Economics, Business and Accounting. 2023;23(21):197-207.
Available:https://doi.org/10.9734/ajeba/2023/v23i211127
70. Olaniyi FG, Olaniyi OO, Adigwe CS, Abalaka AI, Shah NH. Harnessing predictive analytics for strategic foresight: A comprehensive review of techniques and applications in transforming raw data to actionable insights. Asian Journal of Economics, Business and Accounting. 2023;23(22): 441-459.
Available:https://doi.org/10.9734/ajeba/2023/v23i221164

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