INVESTIGATION OF ELECTROCATALYTIC PROPERTIES OF LACCASE AND THEIR MIMICS

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by

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To the unwavering pillars of my support

Maa and Baba

Thank you for believing in me even when I doubted myself

CERTIFICATE

This is to certify that the thesis entitled "**Investigation of electrocatalytic properties of laccase and their mimics"** being submitted by **Ms Bipasa Dey** to the Indian Institute of Technology Delhi for the award of the degree of ***Doctor of Philosophy*** in chemistry is a record of Bonafide research work carried out by her. Ms Bipasa Dey has worked under my guidance and supervision and has fulfilled the requirements for the submission for the thesis, which, to my knowledge has reached the requisite standard. The results contained in this dissertation have not been submitted in part or full to any other university or institute for the award of any degree or diploma.

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Place: New Delhi

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"The real success in education is not just in acquiring knowledge, but in developing the capacity to make the world better through that knowledge."
-Sri Sri Thakur Anukul Chandra

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Bipasa Dey

ABSTRACT

Laccase (EC: 1.10.3.2) is quintessential multi copper oxidoreductase enzyme that can catalyse oxidation of various phenolic and non-phenolic substrates. These enzymes are considered versatile and excellent biocatalyst, with myriads of industrial applications in the domains of textile, pharmaceuticals, food, paper and pulp industries etc. At the catalytic core of the enzyme, copper centres conjugated with conserved network of amino acid residues at two different sites namely T_1 site and T_2/T_3 trinuclear cluster (TNC) serves as a platform for electron transfer, promoting catalytic oxidation–reduction reactions. Such intricate network is instrumental in harnessing the electrochemical capabilities of the enzyme. One of the trademark attributes of laccase is substrate oxidation with concomitant reduction of molecular O_2 into water. This characteristic property renders it suitable for biofuel cells, where direct oxygen reduction is a critical process. High redox potential of a catalyst is often required for a spontaneous oxygen reduction in biofuel cells, which apparently dispensed by fungal laccase more often. However, over the years, the research emphasis has shifted imperatively from eukaryotic to prokaryotic sources for laccase. Despite the low redox potential of the latter, the exceptional stability and enhanced physicochemical properties of bacterial laccases makes them superior alternative to the fungal ones for commercial use. Unlike the traditional inorganic/organic catalysts, laccases are more lucrative as a biocatalyst because they confer various advantages, including high efficiency, high turnover rates and operational stability under mild physiological conditions. Yet, the low redox potential and the instability of the bacterial and fungal laccases respectively, remains the major bottlenecks in this domain. Additionally, the critical limiting factors of reusability, retrieval and storage/operational stability impede the commercialisation of enzymes in general. Therefore, in our study, we attempted to overcome the constraints of critical limiting factors involved in enzyme commercialization by improvising immobilisation strategy. Furthermore, we identified and characterised novel bacterial laccase and investigated their electrocatalytic properties towards oxygen reduction. By practicing the protein engineering approaches, either rational design or directed evolution in conjunction with computational tools, we endeavoured into enzyme modification towards enhance redox potential. Furthermore, by comprehending the core catalytic structure of laccase, we also investigated the potential of copper-based functional mimic of laccase towards electrocatalytic activity.

सारांश

लैकस (EC: 1.10.3.2) एक अत्यंत महत्वपूर्ण मल्टी-कॉपर ऑक्सीडोरिडक्टेस एंजाइम है, जो विभिन्न फिनोलिक और गैर-फिनोलिक सब्सट्रेट्स के ऑक्सीकरण को उत्प्रेरित कर सकता है। इन एंजाइमों को बहुउद्देश्यीय और उत्कृष्ट बायोकैटेलिस्ट माना जाता है, जिनका वस्त्र, फार्मास्यूटिकल्स, खाद्य, कागज और पल्प उद्योगों जैसे अनेक उद्योगों में उपयोग किया जाता है। एंजाइम के उत्प्रेरक केंद्र में, दो अलग-अलग साइट्स पर मौजूद कॉपर केंद्र (T1 साइट और T2/T3 ट्रिन्यूक्लियर क्लस्टर (TNC)) का संरक्षित अमीनो एसिड नेटवर्क इलेक्ट्रॉन ट्रांसफर के लिए एक मंच के रूप में कार्य करता है, जिससे उत्प्रेरक ऑक्सीकरण-अपचयन प्रतिक्रियाएं संपन्न होती हैं।

एंजाइम की इस जटिल संरचना का उपयोग इसकी विद्युत रासायनिक क्षमता को बढ़ाने में होता है। लैक्सेस की एक प्रमुख विशेषता यह है कि यह सब्सट्रेट का ऑक्सीकरण करता है और साथ ही आणविक ऑक्सीजन (O2) को पानी में बदल देता है। यह गुण इसे बायोफ्यूल सेल्स के लिए उपयुक्त बनाता है, जहां प्रत्यक्ष ऑक्सीजन अपचयन एक महत्वपूर्ण प्रक्रिया है। बायोफ्यूल सेल्स में ऑक्सीजन अपचयन के लिए अक्सर उच्च रेडॉक्स क्षमता की आवश्यकता होती है, जिसे प्रायः फंगल लैक्सेस पूरा करता है।

हालांकि, वर्षों में शोध का ध्यान यूकैरियोटिक स्रोतों से हटकर प्रोकारियोटिक स्रोतों की ओर स्थानांतरित हो गया है। भले ही बैक्टीरियल लैक्सेस की रेडॉक्स क्षमता कम हो, लेकिन उनकी उत्कृष्ट स्थिरता और भौतिक-रासायनिक गुण उन्हें व्यावसायिक उपयोग के लिए फंगल लैक्सेस से बेहतर विकल्प बनाते हैं। पारंपरिक अकार्बनिक/कार्बनिक उत्प्रेरकों के विपरीत, लैक्सेस अधिक लाभप्रद बायोकैटेलिस्ट हैं क्योंकि वे उच्च दक्षता, उच्च टर्नओवर दर और सौम्य भौतिक स्थितियों में स्थिरता प्रदान करते हैं।

फिर भी, बैक्टीरियल और फंगल लैक्सेस में कम रेडॉक्स क्षमता और अस्थिरता क्रमशः इस क्षेत्र में प्रमुख बाधाएं बनी रहती हैं। इसके अलावा, पुनः उपयोग, पुनर्प्राप्ति और भंडारण/संचालन स्थिरता जैसे महत्वपूर्ण सीमित कारक एंजाइमों के व्यावसायीकरण में बाधा डालते हैं।

इसलिए, हमारे अध्ययन में, हमने एंजाइम व्यावसायीकरण में शामिल महत्वपूर्ण सीमित कारकों को दूर करने के लिए इममोबिलाइजेशन रणनीति को सुधारने का प्रयास किया। इसके अतिरिक्त, हमने नए बैक्टीरियल लैक्सेस की पहचान और विशेषता की और उनके ऑक्सीजन अपचयन की विद्युत उत्प्रेरक गुणों की जांच की। प्रोटीन इंजीनियरिंग दृष्टिकोण, जैसे कि रेशनल डिज़ाइन या निर्देशित विकास (डायरेक्टेड इवोल्यूशन) को कम्प्यूटेशनल टूल्स के साथ उपयोग करके, हमने एंजाइम के रेडॉक्स क्षमता को बढ़ाने के लिए इसके संशोधन का प्रयास किया। इसके अलावा, लैक्सेस की कोर उत्प्रेरक संरचना को समझते हुए, हमने लैक्सेस के कॉपर-आधारित फ़ंक्शनल मिमिक की विद्युत उत्प्रेरक गतिविधि की भी जांच की।

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LIST OF ABBREVIATIONS

ABTS	2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid
BSA	Bovine serum albumi
BLAST	Basic Local Alignment Search Tool
CD	Circular Dichroism
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
kDa	Kilo Dalton
ATCC	American type culture correction
LB	Luria Bertani
MTCC	Microbial Type Culture Collection
RPM	Rotation per minute
SDS	Sodium dodecyl sulfate
SDS PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
DLS	Dynamic light scattering
TEM	Transmission electron microscopy
SGZ	Syringaldazine
TEMED	Tetramethylethylenediamine
TGA	Thermogravimetry analysis
FTIR	Fourier transform infrared spectroscopy
SD	Standard deviation