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NITRATE REDUCTASE- A NOVEL CATALYTIC ENZYME

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ABSTRACT

A key enzyme in plants, algae, fungi, and some bacteria is nitrate reductase (NR), which catalyzes the reduction of nitrate (NO3-) to nitrite (NO2-), an essential step in the assimilation of nitrogen. The transformation of inorganic nitrogen into forms that living things can employ to create proteins and other nitrogen-containing molecules depends on this process. In addition,nitrate reductase (NR) is mainly used in nitrogen metabolism, environmental remediation, nano biosynthesis and as a biochemical tool.

KEY WORDS: Nitrate; Enzyme; Plants; Microbes;Catalytic efficiency; Biosynthesis.

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INTRODUCTION

Together with nucleic acids and other biological components, nitrogen forms the basis of proteins, making it one of the essential components of life [1]. Because atmospheric N_2 nitrogen exists in an unusable state, most organisms are unable to use it [2]. In order to meet their nitrogen needs, all plants and microbes need nitrogen molecules like nitrate ($NO3^-$) [3]. Nitrate reductase is a necessary first step in the nitrate absorption process that is used by plants, fungus, and some bacteria. Nitrate reductase enables organisms to transform nitrogen into organic compounds by reducing nitrate to nitrite [4]. Because of its catalytic efficiency, which facilitates environmental sensing and biosynthesis, researchers use this enzyme to explore technological applications in nanobiotechnology [5]. By acting as an oxidoreductase, the enzyme NR converts $NO3^-$ to $NO2^-$ [6]. The synthesis of organic nitrogen compounds, which lead to amino acids, proteins, and other nitrogen-based biomolecules, is made possible via biological transformation via nitrate reduction [7]. Because it controls nitrogen cycling in systems that require more nitrogen, NR is crucial to industrial output [8]. When exposed to the right amount of carbon and the right amount of light, the enzyme in plants responds to the availability of nitrate by becoming more active and resilient to stress [9].Nitrate reductase's reduction process requires either NADH or NADPH as an electron donor [10].

The following is the whole chemical process that NR catalyzes:$NO^-3 + 2H+ + 2e^- \rightarrow NO^-2 + H2O$

DISCOVERY

Early in the 20th century, plant physiological studies of crop nitrogen metabolism led to the discovery of nitrate reductase enzymes. It was discovered that plants could absorb nitrate and use it to make beneficial nitrogenous compounds [11]. Only in the 1940s and 1950s did scientists discover that nitrate reductase was the assimilatory enzyme [12]. To identify and investigate its biochemical characteristics, the researchers combined spectrophotometric testing with enzyme extraction techniques. Over time, scientific research has revealed that many NR isoforms and regulatory mechanisms have been found in a variety of microbes [13]. The successful cloning and expression of NR genes by scientists using molecular biology and genetic engineering techniques has increased our understanding of its structure-function design and technological applications [14].

MECHANISM

Nitrate reductase uses a number of cofactors that operate in successive steps to carry out its electron transfer mechanism. Nitrite reduction results from the enzyme's use of cofactors to move electrons from NADH to the nitrate ion [15]. Among the primary cofactors are:

- Electrons are taken up by Flavin adenine dinucleotide (FAD) from NADH/NADPH.
- Electrons are transferred within the enzyme via the heme (cytochrome b557)

- Through the participation of the molybdenum cofactor (MoCo), nitrate is reduced to nitrite [16, 17].

The cytosolic area of plant and fungal cells is where the enzyme functions, and a variety of internal and external factors regulate its expression. The regulation of NR gene expression and the activity of its related enzyme is influenced by light exposure, nitrogen feeding sources, and cellular energy state [18].

SOURCES

A wide range of biological samples contain nitrate reductase including:

- **Plants:**Spinacia oleracea (Spinach), Arabidopsis thaliana, Zea mays (Maize) etc [19]
- **Fungi:**Aspergillus niger, Fusarium oxysporum etc [20]
- **Bacteria:**Pseudomonas spp, Bacillus spp etc [21]

Recombination of nitrate reductase can be produced by current biotechnology thanks to bacterial and yeast expression methods. This method helps the production process by producing and purifying huge quantities of material that can be used in industry and for research purposes [22].

ROLE IN PLANT PHYSIOLOGY

In plant physiology, nitrate reductase serves as the primary doorway for the enzymatic process of nitrate assimilation. The ability of a plant to process available nitrogen determines how efficiently it uses nitrogen, which has an impact on crop output, growth, and productivity [23]. Higher photosynthetic rates, better root and shoot growth, and greater resilience to stressors are all directly correlated with NR activity level [24]. Growth limitations and symptoms of nitrogen deficit are caused by an excessive NR deficiency [25]. Post-translational pathways, transcriptional, and translational mechanisms all work together to control the activity of NR in plants. Light signals are necessary for the synthesis of NR mRNA, while photosynthetic sugars provide the energy needed for the nitrate assimilation process [26]. Drought and salinity stress are two ethological phenomena that have been found to have an impact on NR activity, which in turn produces an enzyme that is useful for evaluating the health of plants [27].

ROLE IN NANOBIOTECHNOLOGY

The field of nanobiotechnology employs nitrate reductase in a variety of creative ways.

- **Green Nanoparticle Synthesis:**Silver ions (Ag+) and gold ions (Au3+) are reduced by the enzyme NR to produce AgNPs and AuNPs. The enzymatic method eliminates the need for hazardous chemical reduction reagents and is a green and environmentally safe way to produce nanoparticles [28].
- **Biosensors:** Following attachment onto carbon nanotubes or gold nanoparticles, NR is used as a biological identification element in biosensors. These sensors report nitrate levels during environmental assessments and agricultural operations, making it feasible to derive information about the nitrate content of soil or water [29].

- **Environmental Cleanup:** NR-containing research-based nanotechnological materials are used to extract nitrates from contaminated water sources. Through the prevention of nitrate overload and the ensuing algal blooms that reduce oxygen levels in aquatic environments, the use of nanomaterials prevents eutrophication [30].

FACTORS AFFECTING NITRATE REDUCTASE ACTIVITY

In addition to physiological factors, a number of environmental factors can influence nitrate reductase activity.

- **Light:** Encourages photosynthetic organisms to express the NR gene [31].
- **Availability of nitrate**: serves as NR induction's substrate and signal [32].
- NR is more active when sugars produced by photosynthetic processes are present [33].
- The optimal level of NR activity is obtained when solutions are kept at neutral pH levels and between 25 and 30°C [34].
- Heavy metal exposure, drought stress, and salinity stress all reduce NR expression while obstructing its ability to function [35].

Understanding these regulating factors advances biotechnology and increases the efficiency of agricultural N use.

NITRATE REDUCTASE USE IN PAKISTAN

Because of its critical significance in crop cultivation, environmental sustainability, and the development of nanobiotechnology, nitrate reductase research is progressing in Pakistan. The use of nitrate reductase as a tool for crop improvement and the creation of green nanotechnology is being studied by a number of universities and research organizations [36]. Research on producing silver nanoparticles (AgNPs) using nitrate reductase from indigenous fungal strains was conducted by the National Institute for Biotechnology and Genetic Engineering (NIBGE), located in Faisalabad. Because of their antibacterial properties and potential for managing plant diseases in agriculture, researchers are interested in these nanoparticles [37]. The University of Karachi and Quaid-i-Azam University in Islamabad do research on the production of NR-mediated nanoparticles for environmental preservation and water purification. The goal of the project is to develop affordable, sustainable nanomaterials that will address the problem of nitrate pollution in agricultural drainage water [38]. In order to monitor water and soil nitrates in real time, COMSATS University and PCSIR research teams are collaborating to develop biosensor devices by combining NR-based biosensors with nanomaterials. Precision agriculture and sustainable farming both depend on these technological advancements [39]. In order to achieve significant nanobiotechnological advancements through collaborative research efforts, Pakistani scientists use the Birch-NR enzyme as a biotechnological catalyst [40].

CONCLUSION

Thus, Nitrate reductase holds significant importance because of its catalytic efficiency and crucial role in nitrogen metabolism.

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