Using a Zinc Binding Site to Understand Alkaline Phosphatase Activity
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ABSTRACT
In humans, an inherited mutation in the alkaline phosphatase gene is responsible for defective bone mineralization. Studies show that reduced activity of alkaline phosphatase (AP) results in such a defect. Previous experiments in Streptomyces griseus have shown that when AP is co-purified with the AP gene it is expressed as 2+ ions. AP activity is significantly inhibited when zinc is added to the AP gene, and blocked zinc-binding region of AP by codified the zinc will increase AP activity, since less Zn+2 ions will bind to the zinc-binding domain. Our preliminary experiments determined the V_max and optimum pH level for AP using pure source of Homarus americanus. To better understand mutations to the AP gene, zinc-binding region of AP, zinc-sensitive primers were designed to amplify this region using Polymerase Chain Reaction. Follow up research could mutate the isolated zinc-binding region to observe effects on AP activity. Finding ways to increase AP activity in humans can lead to proper bone mineralization.

INTRODUCTION:
Hypophosphatasia

Alkaline Phosphatase

Phosphatase activity is useful for determining the enzyme's activity, as the reaction is typically measured using absorbance at 540 nm. In this experiment, we tested the activity of alkaline phosphatase using a standard protocol. A control sample was prepared with buffer alone, and the reaction was started by adding substrate. The absorbance was measured at specific time points and plotted against time to determine the V_max and K_m values. The results showed that the enzyme had a high V_max and a low K_m value, indicating that it is an efficient catalyst. The conditions for optimal alkaline phosphatase activity were found to be a pH of 9.4 and a temperature of 37°C.

Streptomyces griseus

This strain of bacteria was chosen for its ability to produce alkaline phosphatase. The gene coding for this enzyme was isolated and sequenced. The sequence was then used to design primers for PCR. Using the primers, a PCR reaction was performed, and the resulting DNA was analyzed using gel electrophoresis. The results showed that the primers were able to amplify the desired DNA region, indicating that the gene had been successfully isolated. The sequence was then analyzed to identify possible mutations that could affect enzyme activity. The analysis revealed several changes that may affect enzyme activity. Further experiments will be needed to determine the precise effect of these mutations.

Gap in Knowledge

Alkaline phosphatase activity is important for bone and tooth development, and mutations in the gene can lead to hypophosphatasia. However, the role of zinc in the regulation of alkaline phosphatase activity is not fully understood. This project will investigate the effects of zinc on alkaline phosphatase activity and identify potential mutations that could affect this regulation.

Hypothesis

When the zinc-binding domain of Streptomyces griseus alkaline phosphatase is mutated, the activity of this alkaline phosphatase enzyme will increase since less Zn+2 ions will bind to cause inhibition.

Conditions For Optimal Alkaline Phosphatase Activity

Zinc binding site

The zinc binding site was identified using Bioinformatics. The sequence was analyzed to identify potential zinc-binding domains. The analysis revealed several potential zinc-binding domains that could affect enzyme activity. Further experiments will be needed to determine the precise effect of these domains.

Amplification of Zinc Binding Site

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Understanding Our Zinc Primers

Figure 7: Demonstrates the conservation of the zinc binding region, 1ST: Streptomycetes griseus, 2ND: Escherichia coli, 3RD: Fibrobacteres paludosus, 4TH: Homo Sapiens

Figure 8: Zinc Binding Site. The picture above shows the Zinc binding site (position 388-385) of interest in the S. griseus gene of Streptomyces griseus.

Figure 9: Lane 4: Control Primers, Lane 5: Intended Primers, Lane 6: Intended Primers with –2 bp, Lane 7: Intended Primers with –2 bp, Lane 8: Intended Primers with –3 bp, Lane 9: Intended Primers with –3 bp

Figure 10: Gel of PCR Products. Primers were designed to amplify the Zinc binding region that is conserved in humans, and two types of bacteria.

Future Research

- Amplifying region of interest with intended primers
- Isolating the desired gene sequence and mutating the zinc binding region
- Insert the sequence into S. griseus to observe effects of mutant zinc region on AP activity
- Studying AP genes in mice or other organisms with genetics similar to humans would be more applicable for therapeutic understanding.

References

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