Quadrant II – Transcript and Related Materials

Programme: Bachelor of Science (Third Year)

Subject: Zoology

Paper Code: ZOD 104

Paper Title: Animal Biotechnology

Unit: 3

Module Name: Polynucleotide Kinases and Alkaline Phosphatases

Module No: 25

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NOTES

There are numerous enzymes that modify the DNA molecules either by addition or removal of a specific chemical groups from the DNA strands. This DNA is used in various genetic engineering techniques.

Some of the important DNA modifying enzymes are as follows:

1. ***Polynucleotide kinase*** (from E. coli infected with T4 phage), which adds phosphate groups onto free 5' termini.
2. ***Alkaline phosphatase*** (from E. coli, calf intestinal tissue or arctic shrimp), which removes the phosphate group present at the 5' terminus of a DNA molecule.

The enzymes polynucleotide kinase and alkaline phosphatases acts on the termini of DNA molecules. They provide an important function to DNA in the field of biotechnology.

1) POLYNUCLEOTIDE KINASE

Polynucleotide Kinase is a homotetramer of 301 amino acid polypeptides. This homotetramer is formed by the formation of homodimer of phosphatase-phosphatase and kinase-kinase.

This enzyme is obtained from *E coli* infected by T4 bacteriophage. It has a phosphorylating activity. It catalyses the transfer and exchange of a phosphate group from the ATP (adenine ribose triphosphate nucleotide) to the 5' hydroxyl terminus of double stranded or a single stranded DNA or RNA. This enzyme is also known to remove 3' phosphoryl groups. Phosphate transfer activity is optimum at 37°C, pH 7.6, Mg2+ and reducing reagents

Polynucleotide Kinases shows both phosphorylation as well as phosphatase activities. The enzymatic activity of Polynucleotide Kinases is utilized in two types of reactions i.e., **Forward reaction and Exchange reaction**.

1. ***FORWARD REACTION:***

   In the forward reaction a phosphate group is added at the 5' end of the DNA. Polynucleotide Kinase transfers the gamma phosphate from ATP to the 5' end of DNA or RNA strand.

2. ***EXCHANGE REACTION:***

   In the exchange reaction the target DNA or RNA that has a 5' phosphate is incubated in presence of excess of Adenosine di- phosphate and Polynucleotide kinase. The polynucleotide kinase will first transfer the phosphate from the nucleic acid onto an ADP forming ATP and leaving a dephosphorylated target. And then it will perform a forward reaction and transfer a phosphate from ATP onto the target nucleic acid.

Applications of Polynucleotide Kinase:

- Polynucleotide is used for radiolabeling oligonucleotides, usually with 32P, for use as hybridization probes.
- Since polynucleotide kinase has a phosphorylating activity, it is used to phosphorylate linkers and adaptors, or fragments of DNA as a prelude to ligation.
- Polynucleotide kinase is widely used to end label short oligonucleotide probes, DNA or RNA molecules.

- The activity of Polynucleotide Kinase is inhibited by small amounts of ammonium ions, phosphate ions, or NaCl.
- This enzyme also helps in removal of 3’ phosphoryl groups.

2) ALKALINE PHOSPHATASE

Alkaline Phosphatase is a homodimeric enzyme that catalyses the reactions like hydrolysis and transphosphophorylation. Alkaline phosphatase is most active at alkaline pH (10). Hence the name alkaline phosphatase. It is also known as basic phosphatase. This enzyme catalyzes the removal of 5' phosphate groups from RNA, DNA and ATP. Alkaline phosphatase is used to prevent unwanted ligation of DNA molecules.

Treatment of DNA with Alkaline Phosphatase prevents self-ligation. Self-ligation can be problem in certain cloning procedures. Ligation only takes place between 3’ and 5’, when 5’ is phosphorylated. During ligation of desired insert, the complementary ends of the insert and vector will come to proximity of each other (only for sticky ends but not for blunt ends). In order to overcome this problem of re-circularisation, the restricted plasmid is treated with alkaline phosphatase.

TYPES OF ALKALINE PHOSPHATASE

1. ***Calf intestinal alkaline phosphatase (CIP)***
   It is isolated from calf intestine. This enzyme catalyzes the removal of phosphate group from 5’ end of DNA as well as from RNA.
2. ***Shrimp alkaline phosphatase (SAP)*** – it is a highly specific and heat labile phosphatase enzyme. It is isolated from arctic shrimp (*Pandalus borealis*). It removes 5’ phosphate group from DNA, RNA, deoxyribonucleotide triphosphates and proteins. Shrimp Alkaline Phosphatase can be irreversibly inactivated by heat treatment at 65°C for 15mins.

Alkaline phosphatase is also classified into four isozymes based upon the tissue that synthesises alkaline phosphatase.

APPLICATION OF ALKALINE PHOSPHATASES

This enzyme is used in removal of 5’ phosphate from different vectors like plasmid and bacteriophage vectors after treating them with restriction enzyme.

Treatment with Alkaline Phosphatase precents self-ligation of the vector.

It is used to remove the 5’ phosphate from fragment of DNA prior to labelling with radioactive phosphate.

It facilitates ligation of other DNA fragments into the vector, where one strand of the insert having 5’-phosphate will ligate with the 3’OH of the vector.

This nick will then be sealed by DNA ligase enzyme in the presence of ATP.