ANALYSIS OF THE REPAIR ACTIVITY OF ENZYMES INVOLVED IN THE BASE CLEAVAGE REPAIR PATHWAY

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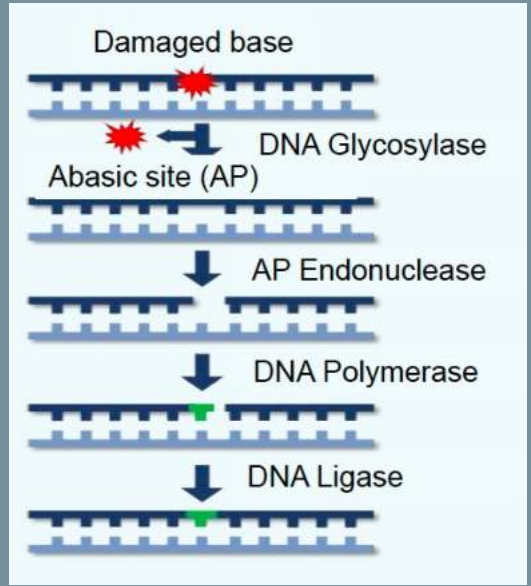
INTRODUCTION

The Base Excision Repair pathway is a crucial mechanism for the maintenance of genome stability. The BER (Base Excision Repair) mechanism consists of several steps and is initiated by the action of a specific class of repair enzymes called DNA Glycosylases, which catalyse the hydrolysis of the N-glycosyl bond linking the altered base to the sugar-phosphate backbone.

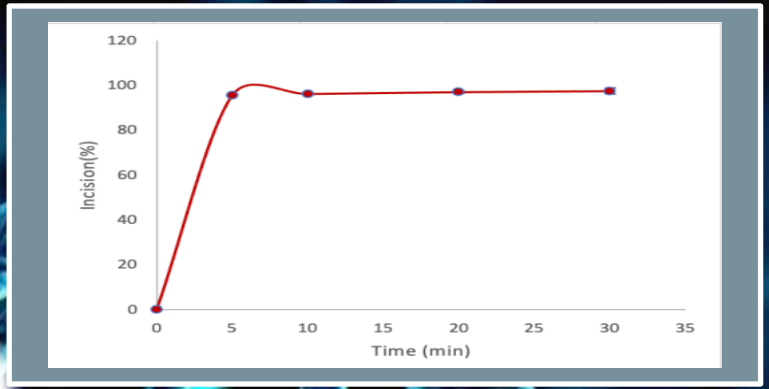
There are different types of DNA Glycosylases, each of which recognises a specific type of lesion or range of chemically related lesions. After this initial step, which releases the damaged base as a free base, an abasic site (AP) is generated. This AP site is processed by an endonuclease that catalyses the hydrolysis of the phosphodiester bond to the 5' side of the base-free residue, generating a 3'-OH end that will be used at a later stage of repair. In addition, a 5' end is produced with a deoxyribose phosphate residue without base (dRP), which is removed by the action of a deoxyribose phosphate diesterase (dRPase).

Finally, continuity of the damaged strand is restored by the action of a DNA polymerase and a ligase.

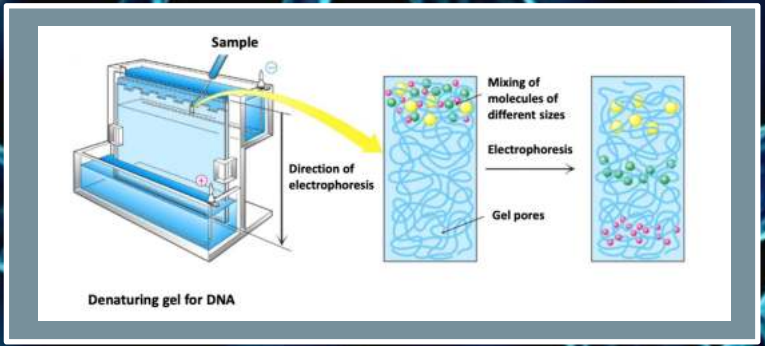


BASIC CONCEPTS

ENZYMATIC KINETIC

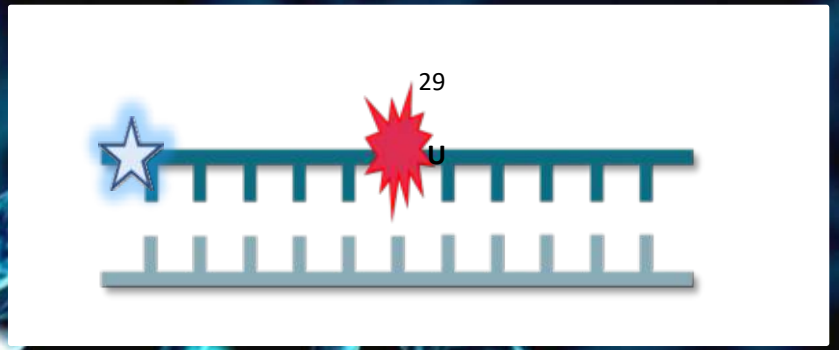


ELECTROPHORESIS

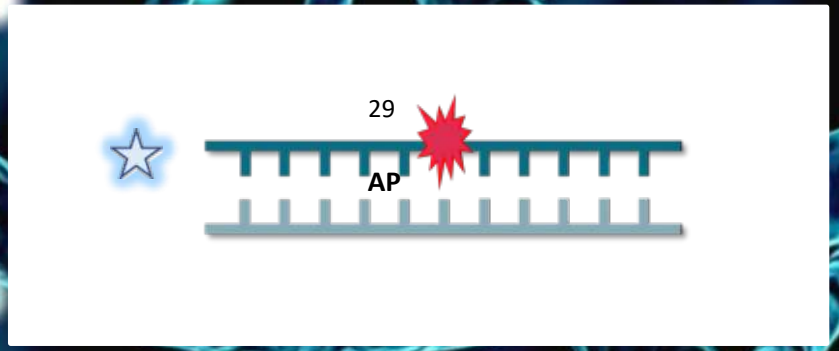


BASIC CONCEPTS

URACIL DNA GLYCOSYLASE (UDG)



HUMAN AP ENDONUCLEASE 1

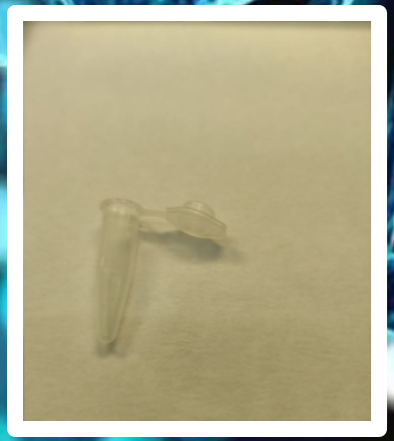


RESEARCH OBJECTIVES

To analyse the repair activity of two enzymes, Uracil DNA glycosylase and Human AP endonuclease 1 at different times.

To obtain two enzyme kinetics curves, one for each enzyme as a function of the times used.

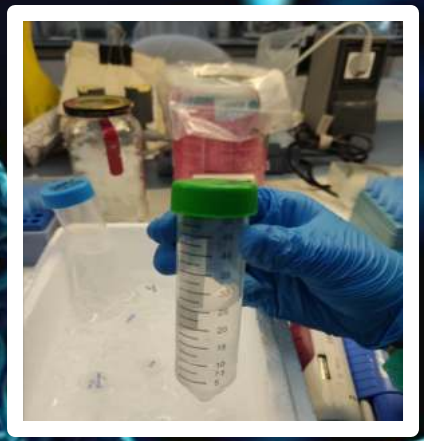
MATERIALS



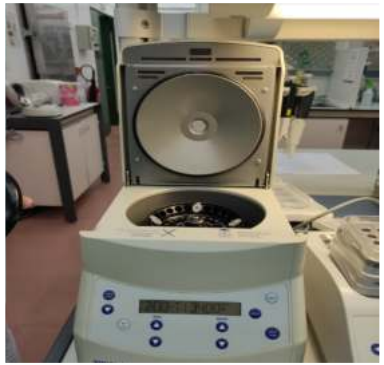
EPPENDORF TUBES

MICROPIPETTE

SKIRTED FALCON TUBES

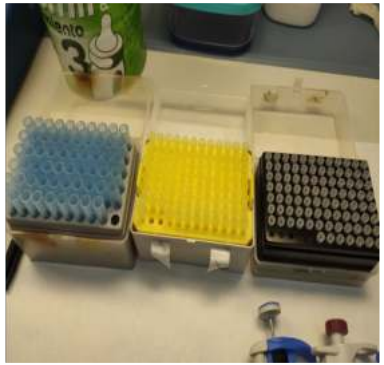


SKIRTLESS FALCON TUBE



CENTRIFUGE

THERMOSHOCK



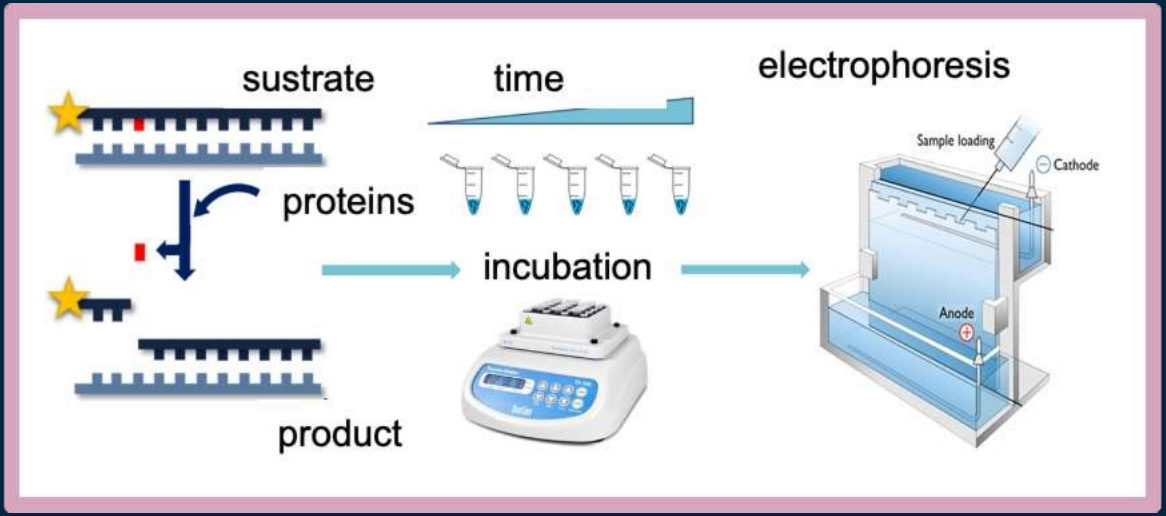
PIPETTE TIPS

AUTOCLAVE

BUCKETS WITH CRUSHED ICE

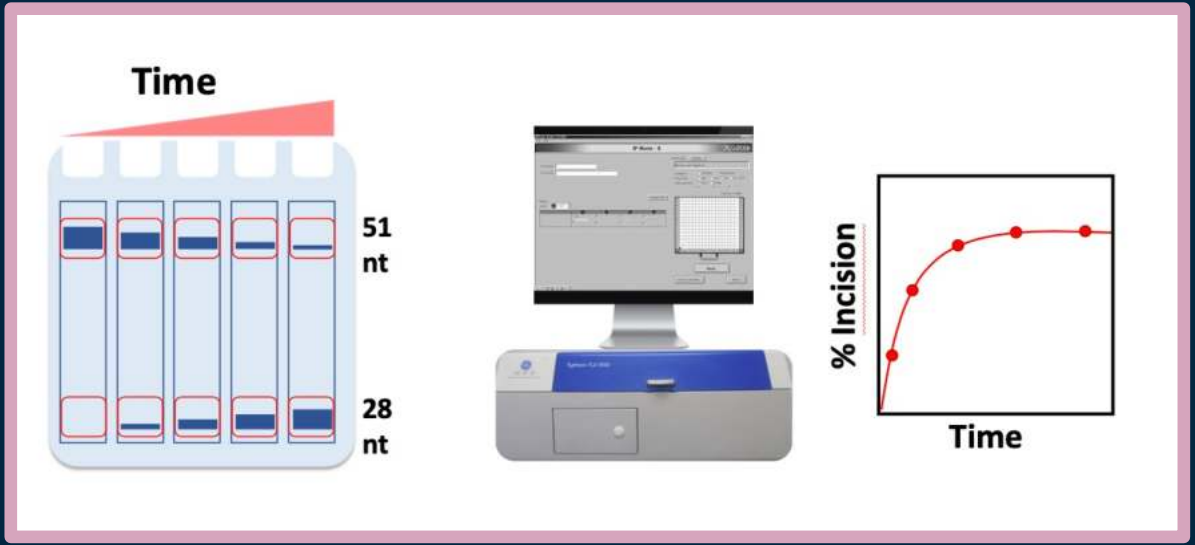
METHODS

DNA substrates damaged by a uracil or a basic site are synthesised and fluorescently labelled. Subsequently, enzyme activity assays were performed with the enzyme AP Endonuclease and DNA Glycosylase, analysing the efficiency by the appearance of product at different times of the enzymatic reaction. After the reaction, DNA was purified and electrophoresis was performed on acrylamide gels.

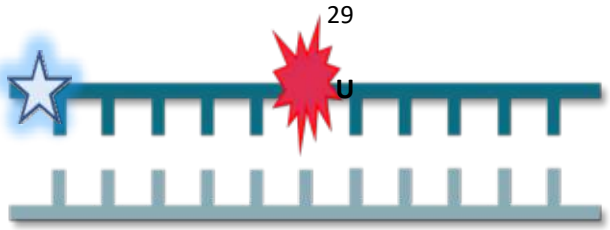


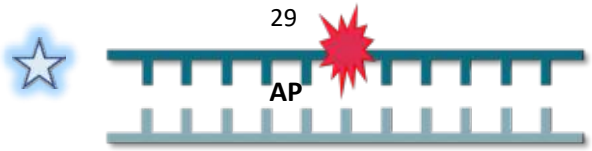
METHODS

After electrophoresis, the data were revealed using a fluorescence scanner and subsequently analysed and quantified using Multigauge software to obtain the enzyme kinetics. Finally, the unprocessed substrate of 51 nt and the accumulation of processed product of 28 nt with respect to time was observed.



RESULTS





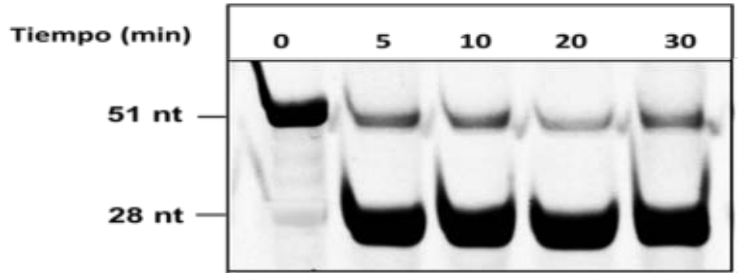
URACIL DNA GLYCOSYLASE (UDG)

First, 20 nM of damaged DNA substrate was incubated with a uracil at position 29 in the presence of Uracil DNA Glycosylase (UDG) 0.2 U at 5, 10, 20 and 30 minutes.

HUMAN AP ENDONUCLEASE

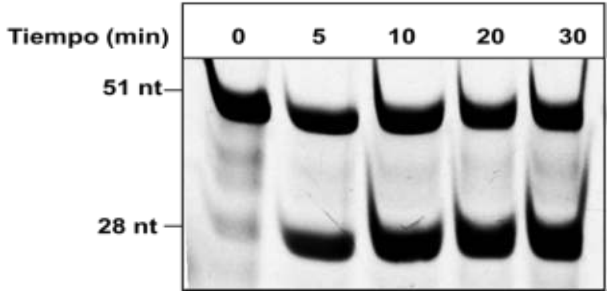
In addition, Human AP endonuclease 1 0.2U activity was analysed using substrates (20 nM) that were damaged by an abasic site at position 29 at 5, 10, 20 and 30 minutes.

RESULTS



ELECTROPHORESIS
URACIL DNA GLYCOSYLASE (UDG)

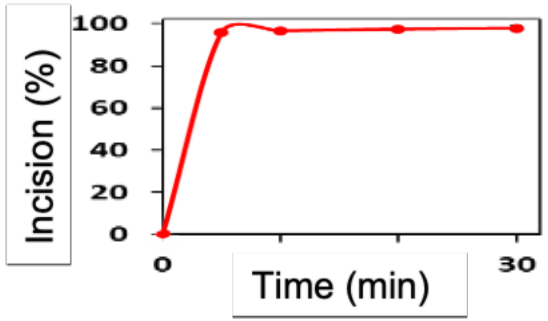
Image shows the results of the electrophoresis where the process carried out by the enzyme Uracil DNA Glycosylase is revealed, being able to observe the unprocessed 51nt substrate and the accumulation of processed 28nt product with respect to time.
As can be seen, Uracil DNA Glycosylase is highly efficient, since all the substrate is processed after the first 5 minutes.

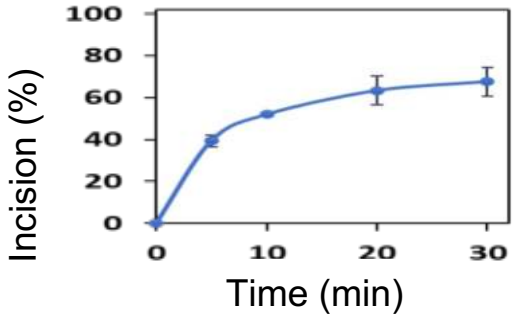


ELECTROPHORESIS
HUMAN AP ENDONUCLEASE

Image shows the results of the electrophoresis, where the process carried out by the human AP endonuclease 1 enzyme is revealed, being able to observe the unprocessed 51nt substrate and the accumulation of processed 28nt product with respect to time.

RESULTS





ENZYME KINETICS
URACIL DNA GLYCOSYLASE (UDG)

After analysis of the data, the results indicated that this enzyme is highly efficient, reaching 100% of the substrate processed within 5 minutes of the start of the enzymatic reaction.

ENZYME KINETICS
HUMAN AP ENDONUCLEASE

The data was analysed and it was observed that this enzyme presents a lower efficiency than UDG, since after 5 minutes we obtained an incision of 40%.

CONCLUSIONS

The amount of substrate and enzymes used were adequate for the study of the Base Excision Repair (BER) mechanism.

The enzymes studied, Uracil DNA glycosylase and HumanAP endonuclease 1, are able to recognise and process the substrates tested

Uracil DNA glycosylase is more efficient than human AP endonuclease 1.

Uracil DNA Glycosylase in the first 5 minute interval processed 100% of the substrate, while Human AP Endonuclease 1 processed 40% of the substrate.

FINAL CONCLUSIONS

The study of the kinetics of the two enzymes shows that Uracil DNA Glycosylase is more efficient than Human AP Endonuclease 1.
This would indicate that the processing of damaged bases by Uracil DNA Glycosylase would not be a limiting step in the Base Excision Repair pathway (BER).

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