The enzyme-modified comet assay: Measuring oxidized and alkylated bases using the 12-gels format

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The standard alkaline comet assay detects DNA strand breaks and alkali-labile sites at single-cell level. However, many DNA-damaging agents induce other lesions (e.g. oxidized and alkylated bases). This limitation is overcome by the combination of the assay with DNA repair enzymes. Indeed, several enzymes are already being used, but their specificity to detect certain lesions has not been assessed.

The final aim of this work is to study the specificity of several enzymes in order to select the most convenient ones to detect oxidized and alkylated bases. The following commercial enzymes are being used: formamidopyrimidine DNA glycosylase (Fpg), endonuclease III (Endo III), human 8-oxoguanine DNA glycosylase (hOGG1) and human alkyladenine DNA glycosylase (hAAG). For the first time, the hAAG, is being used in combination with the comet assay. The widely used Fpg produced and distributed by NorGenoTech (Norway) (Fpg-NGT) has also been tested.

The differences between the 2 and 12 gel/slide systems were assessed by the titration of Fpg-NGT in both formats and using different times of incubation (37ºC). TK-6 cells treated with potassium bromate ($KBrO_3$) to induce oxidized bases were used. Results showed a different pattern when using different formats.

The rest of the enzymes were titrated using the 12 gels/slide system and 1 hour of incubation. TK-6 cells treated with methyl methanesulphonate (MMS), to induce alkylated bases, were used for hAAG. On the other hand, TK-6 cells treated with $KBrO_3$ were used to titrate the rest of the enzymes. The same reaction buffer was used with all the enzymes which is very convenient if different enzymes are meant to be used at the same time. Results of the titrations will be presented and compared with the manufacturer instructions. Endo III was not successfully titrated; another compound to induce Endo III-sensitive lesions will be tested.

The specificity of the enzymes was also studied on Glyco-SPOT biochips by LXRepair, obtaining the expected results. To complete this work, all enzymes will be tested in TK-6 cells treated with non-cytotoxic concentrations of different genotoxic compounds including MMS and $KBrO_3$. Data showing the ability of hAAG to detect MMS-induced lesion but not $KBrO_3$-induced lesions will be presented.

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