SPR-imaging based assays on an oligonucleotide-array to analyze DNA lesions recognition and excision by repair proteins

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ABSTRACT

An original oligonucleotide-array, coupled with SPR-imaging detection, has been developed to study biological interactions between DNA base lesions and DNA repair enzymes. This bioanalytical tool constitutes an efficient screening platform to quantify DNA repair activities and to search for new DNA repair inhibitors.

INTRODUCTION

Base excision repair (BER) is the major mechanism for correction of damaged nucleobases resulting of alkylations and oxidation to DNA (1,2). The first step in the BER pathway consists in excision of the abnormal base by several specific DNA N-glycosylases. A decrease in BER activity was found related to an increased risk in carcinogenesis and aging (3,4). Therefore, the correct functioning of DNA repair machinery is crucial for the maintenance of the genomic integrity and cell viability. To investigate BER activities we set up a new oligonucleotide-array for DNA repair analysis based on surface plasmon resonance imaging (SPRI). The latter label-free DNA sensorchip permits not only to detect N-glycosylase/AP-lyase activities, but also the binding of repair proteins to DNA damage without cleavage activity.

![Figure 1: Structure of the damaged nucleosides site-specifically inserted into oligonucleotides and used in the SPRI-based enzymatic repair assays.](https://example.com/figure1.png)

RESULTS AND DISCUSSION

Modified oligonucleotides bearing an oxidatively generated base damage, namely 8-oxo-2'-deoxyguanosine (8-oxo-dG) and (5'S) 5',8-cyclo-2'-deoxyadenosine (5',8-cyclo-dA) (Figure 1), were prepared by solid-phase chemical synthesis as previously described (5, 6). These damaged base-containing DNA fragments were hybridized with complementary strands grafted by a pyrrole electropolymerisation process on a glass prism coated with a gold layer (7). Then, the resulting lesion-containing oligonucleotide-array coupled with surface plasmon resonance imaging (SPRI) detection, was used to investigate the behavior of the E.coli Fapy DNA N-glycosylase protein (Fpg), a repair enzyme involved in the processing of oxidized nucleobases (8).

First, by using a thermal denaturation procedure followed by a specific hybridization step (Figure 2) the latter enzyme was shown as expected to bind and then cleave its natural substrate, namely 8-oxo-7,8-dihydroguanine, together with the resulting abasic site.

![Figure 2: Fpg DNA repair activity can be observed using specific temperature denaturation. After 8-oxodG recognition, there is a reaction cascade including excision of the damaged nucleotide by Fpg. Two short strands (7-mer and 14-mer respectively) are formed which quickly denature at 45°C compared to the unmodified duplexes (22-mers). Consequently, after three minutes at 45°C, all the gapped duplexes dissociate and we observe specific hybridization by injecting the complementary sequence on the chip.](https://example.com/figure2.png)

The use of the current DNA biochip also allows the observation of an original binding activity of Fpg protein towards the (5'S) 5',8-cyclo-2'-dAdenosine residue without
an additional AP-lyase activity (Figure 3). Such a behavior should have an important biological significance since the latter oxidative damage constitutes a possible endogenous inhibitor for Fpg protein and other DNA N-glycosylases that consequently decreases the cellular repair capacities. These results altogether show that the present oligonucleotide microarrays coupled with SPR imaging may be used to simultaneously and specifically detect recognition and excision steps of several DNA damages by repair enzymes (9).

CONCLUSION

In the current work, we apply for the first time SPR-imaging to assess DNA N-glycosylase binding and catalytic properties toward various oxidatively damaged DNA sites. The present miniaturized label-free SPRi-based assay should provide a powerful tool to further substantiate the role of repair capacity in the development of cancer and human health. This technique should also be used as screening platforms to search for new DNA repair activity modulators, such as inhibitor drugs used in radio- and chemotherapy protocols.

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