Study on the Application of Furan Crosslinking at the Protein-DNA Interface

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Introduction

The toxicity of furan is known to rely on its selective oxidation in the liver by Cyt P450 enzymes transforming it into the very reactive butenedial, which quickly reacts with proximate nucleophiles of DNA (Figure 1) and proteins. [1] This principle was used in our laboratory to develop a high yielding DNA interstrand crosslinking methodology. [2]

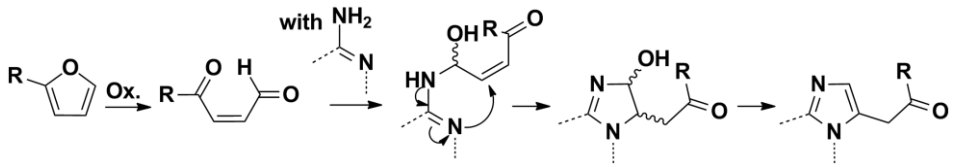

Figure 1. Furan oxidation and reaction with nucleophilic DNA

In view of the demonstrated site-selectivity, the method further holds promise for site-specific crosslinking of proteins to their DNA recognition site, which is highly relevant in the study of transient protein-DNA interactions. Furthermore irreversible DNA binding can be achieved through such a covalent linkage, which is potentially useful for new generation therapeutics. [3]

Results and Discussion

In our previous studies a good major groove binding peptide was found to be prerequisite for such crosslinking experiments to ensure proximity between the reactive enal and the attacking nucleophile. [4] Therefore we chose to work with a miniature transcription factor, to allow for easy modification and analysis. The experiments were carried out with a non-covalent GCN4 mimicking dimer, earlier described by Morii *et al.* (Figure 2). [5]

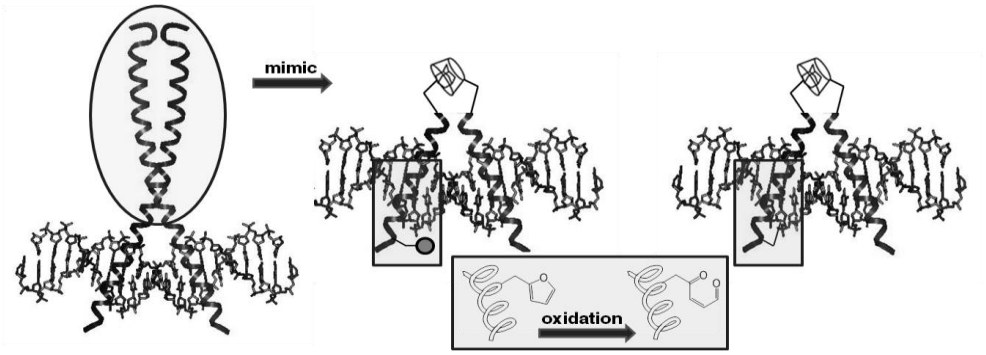

Figure 2. Principle of the crosslinking from furan-modified protein mimic to DNA

Furylalanine was introduced in the peptide by replacing a lysine (K231) or alanine (A239) residue positioned in the major groove, as determined from the crystal structure. [6] Binding of these modified protein mimics was ensured by the generation of heterodimers and verified by EMSA experiments. Optimization of the oxidation conditions was carried out by reaction with hydrazine and monitoring on HPLC. However, furan oxidation in the presence or just before the addition of DNA, did not result in observable crosslink formation. Although unfavorable positioning and/or linking cannot be excluded, we believe that the involvement of the available nucleophilic amine functionalities of the DNA in Watson-Crick basepairing, renders them unreactive towards the crosslinking reaction. This rationale is further supported by the described use of aldehydes for the detection of single stranded regions of DNA [7] and their use to elucidate RNA folding pathways. [8]

We are currently exploring the reverse approach, incorporating the furan moiety into DNA, for further crosslinking to a non-modified protein mimic.

Acknowledgments

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