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Toxicity Inspired Cross-Linking for Probing DNA–Peptide Interactions

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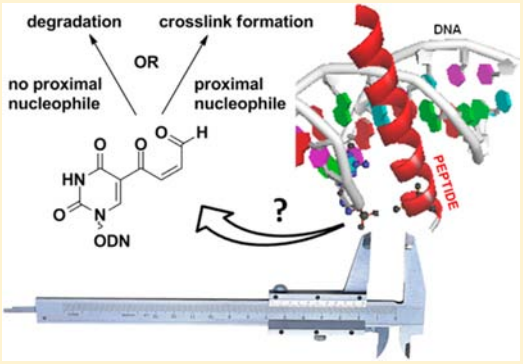
Supporting Information

ABSTRACT: A cross-linking methodology for the study of DNA–protein interactions is described. The method is inspired by the metabolic activation of furans causing toxic DNA damage, including DNA–protein cross-links (DPC). The furan moiety, representing a latent functionality, is easily incorporated into oligonucleotides, and can be activated on demand to release a reactive aldehyde. Reaction with nucleophilic lysine side chains is shown to be distance-sensitive and allows for site-selective DPC formation.



INTRODUCTION

The interaction of proteins with nucleic acids is essential for cellular functioning, as they are responsible for the regulation and execution of many biological processes, including folding, replication, transcription, and degradation. Understanding the remarkable selectivity and affinity of DNA binding proteins for their targets in the complex cellular environment is a major challenge, driven by the ambition to design drugs that can compete in such interactions.[1] In an attempt to decipher the protein–DNA recognition code, analysis of crystal structures and modified proteins has been greatly aided by the use of bifunctional cross-linking moieties, causing covalent locking of the interactions. Photoactivatable functionalities[2−5] such as phenylazides, diazirines, and benzophenones have been exploited for DNA–protein cross-link (DPC) generation, but lack selectivity for identification of the amino acid residues responsible for selective recognition at the DNA–protein binding interface, as the generated reactive species can target any amino acid. Further, due to the occurrence of high-energy intermediates, the resulting structures are not always representative of the actual structure in the absence of the cross-link. Finally, obtained DPC yields are in general rather low (10–20%).[6] In order to probe the DNA–protein interface, use of a cross-linking mechanism requiring the presence of proximal nucleophilic side chain functionalities can be considered as a viable alternative.

Aldehydes, representing sensitive functionalities reactive toward nucleophiles, commonly occur in the environment and are endogenously generated by metabolic activation and lipid peroxidation. As a result of random adduct formation with DNA and proteins, they are mutagenic and cytotoxic, respectively.[7,8] Aldehydes are also known to covalently trap proteins onto DNA exploiting the nucleophilic side chain functionalities of proteins.[9−12] Consequently, externally added formaldehyde is routinely used for random DPC formation in chromatin immunoprecipitation experiments.[13] However, for site-selective formation of a DPC, oligonucleotides (ODNs) containing a suitably positioned aldehyde moiety are required. Abasic sites, which are toxic DNA lesions resulting from oxidative damage that ring-open to aldehydes[14] and can as such subsequently form DPCs, can be locally generated in DNA by action of uracil-DNA glycosylase on a correctly positioned deoxyuracil or by photolysis of a protected precursor.[15,16] Introduction of other aldehyde functionalities into ODNs has been achieved through oxidation of a diol with $NaIO_4$. DPC formation after oxidative conversion of ODNs with diols replacing the sugar[17] or diols attached to the 2′-position[18,19] or the base[20] have been reported. In the latter case 5-formyldeoxyuridine (fdU) is generated, an abundant naturally occurring form of thymidine damage in DNA,[21,22] proposed to be involved in the formation of DPCs.[23,24] Unfortunately the synthesis of these diol building blocks[17−20] with extended protection schemes is lengthy. Aromatic aldehydes can be introduced without protection[25] or through postsynthetic Suzuki-Miyaura coupling with 5-iodocytosine[26] as they are less reactive. However, the lower reactivity is a disadvantage in subsequent cross-linking reactions with nonactivated nucleophiles.

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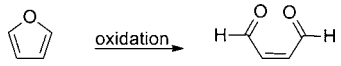
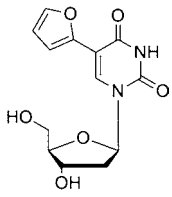
Inspired by the toxic metabolic activation of furan into 4-oxo-butenal (Scheme 1),[27] we report here on the use of easily

Scheme 1. Furan Oxidation



accessible furan-modified ODNs for site-selective DPC formation. The furan moiety represents a latent functionality, which can be oxidatively activated on demand to release the reactive aldehyde. Furan incorporation in ODNs is straightforward as the small aromatic moiety is stable under both nucleotide and ODN synthesis conditions. Moreover, various furan derivatives are commercially available, allowing modular construction of different furan-substituted nucleoside derivatives. We have previously shown that in a nucleic acid context, selective activation by oxidation to generate a reactive aldehyde is possible and can be carried out after duplex formation, thus minimizing nonspecific off-target reactions and causing site selective cross-link formation.[28−31] Due to the obtained high yield and selectivity, the furan-oxidation methodology has recently been accepted as a general procedure to achieve DNA interstrand cross-linking.[32] Additionally, very recently, the usefulness and wider applicability of the furan-oxidation methodology has been illustrated by Summerer and co-workers in the cross-linking of genetically modified proteins incorporating a furan-amino acid toward RNA targets.[33] Here, using nucleoside **1** (Chart 1) rather than our previously developed

Chart 1. Furan Modified Nucleoside 1



(1)

furan modified building blocks, we describe for the first time cross-linking to non-nucleic acid targets, considerably expanding the scope of the furan-oxidation cross-linking methodology and opening the way to a much broader application of furan-based probes.

EXPERIMENTAL SECTION

Materials. NBS, TEAA, and MeCN Chromasolve were purchased from Sigma-Aldrich, $NaCNBH_3$ from Acros Organics. Reagents for DNA synthesis were obtained from Glen Research. Commercial oligonucleotides were purchased, purified over a Gold cartridge from Eurogentec, and used as delivered. The water used in experiments was always Milli-Q grade.

Synthesis of 1. Nucleoside **1** was synthesized through a Stille cross coupling of stannylated furan with commercially available 5-iodo-2′-deoxyuridine. Synthesis, DMTr protection and conversion into the phosphoramidite, was carried out as described in literature ref 42.

Synthesis of 1-Modified ODNs. The modified residue **1** was incorporated into the CRE (5′-CGG ATG ACG TCA TTT TTT TTT TTC-3′) DNA binding site of the transcription factor mimic of GCN4, replacing natural T (CRE1 and CRE2) or natural G (CRE3) residues. ODN synthesis was carried out following the standard procedures for automated ODN synthesis at 1 μmol scales of the DNA synthesizer (ABI 394 DNA-synthesizer). The furan modified nucleoside was introduced by manual coupling with 0.5 mL of 1 M 4,5-dicyanoimidazole and 0.4 mL of 0.06 M of the phosphoramidite building block in dry MeCN, which were applied in alternating portions over the column. Upon completion of the synthesis, the ODN was deprotected and cleaved from the controlled pore glass by overnight reaction in 50 mM NH_4OH solution at 55 °C. Postsynthetic workup and purification was performed using Sep-pak (Waters). Crude yields of over 95% were obtained and samples were used without further purification.

Mass spectra of oligonucleotides were recorded on a quadrupole ion trap LC mass spectrometer (Thermofinnigan, San Jose, Ca, USA) equipped with electrospray ionization in negative mode. Data were deconvoluted using the Agilent LC/MSD ChemStation software. CRE1 ($C_{209}H_{263}N_{67}O_{132}P_{20}$) observed mass 6443.8 Da, calculated mass 6442.1 Da; CRE2 ($C_{209}H_{263}N_{67}O_{132}P_{20}$) observed mass 6443.9 Da, calculated mass 6442.1 Da; CRE3 ($C_{209}H_{264}N_{64}O_{133}P_{20}$) observed mass 6418.15 Da, calculated mass 6417.1 Da. Concentration of ODNs was determined with a Trinean dropsense multichannel spectrophotometer. RP−HPLC analysis was recorded on an Agilent 1100 or 1200 system equipped with a UV diode array detector set at 260 nm, using Phenomenex Clarity (250 × 4.6 mm, 5 μ) or Aeris Widepore (150 × 4.6 mm, 3.6 μ) columns at 60 °C with eluent: 0.1 M TEAA (with 5% MeCN) and MeCN as mobile phase (linear gradient: 0−30% MeCN in 15 or 30 min, 30−100% MeCN in 3 min).

Melting temperature studies were performed using a Varian Cary 300 Bio equipped with a six-cell thermostatted cell holder. The curves were monitored at 260 nm with a heating rate of 0.3 °C/min. A 1 μM sample of 2 complementary ODNs was buffered in 10 mM phosphate buffer of pH 7, with 100 mM NaCl. Melting temperatures were calculated from the first derivative of the heating curves using the Cary 300 Bio software. CRE:CRE comp: 60.7 °C; CRE1:CRE comp: 59.7 °C; CRE2:CRE comp: 60.7 °C; CRE3:CRE3 comp: 57.6 °C.

Synthesis of GCN4 Mimic. The 26-mer GCN4 peptides (DPAALKRARNTEAARRSRARKLQGGC) were synthesized on a PSSM-8 automatic peptide synthesizer from Shimadzu, on PAL PEG PS resin with low loading (0.180 mmol/g, Applied Biosystems) using 10-fold excess of Fmoc (9-fluorenylmethoxycarbonyl) amino acids (Novabiochem), with 10 equiv of HBTU (*O*-Benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate, Novabiochem) as coupling reagent and HOBt (1-hydroxybenzotriazole) as additive from a 1 M solution and with 20 equiv of diisopropylethylamine from a 2 M solution in DMF. Agitation was provided by N_2 bubbling. Single couplings were performed during 30 min. Fmoc deprotection was achieved in double 4 min treatments with 30% piperidine in DMF. The peptide was capped manually with acetic anhydride using *N*-methylimidazole as catalyst. Completion of the reaction was checked by Kaiser test. Final deprotection and cleavage from the solid support was achieved by addition of cold cleavage mixture to the product cooled on ice. The cleavage cocktail consisted of 0.375 g of phenol, 0.5 mL of water, 0.25 mL of thioanisole, 0.125 mL of ethanediol, and 5 mL of TFA. After 15 min, the mixture was allowed to heat up to room temperature and left for 3 h. The pure peptide was obtained by precipitation in cold diethyl ether, which was

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dissolved in water and extracted twice with ether, filtered over a sephadex column, and purified by RP-HPLC.

Modification of the peptides to construct the protein mimic was carried out as described.[40] Adamantane was modified as bromoacetyl and cyclodextrin was monoiodated via a tosylate to allow reaction with the thiol group of the C-terminal cysteine of the GCN4 peptide to obtain Ad-GCN4 and CD-GCN4, respectively. Mass spectra of peptides were recorded on a MALDI TOF (Shimadzu, Kratos analytical, AXIMA-LNR), with α-cyano-4-hydroxycinnamic acid matrix and internal mass standards (Applied Biosystems). Ad-GCN4 ($C_{130}H_{228}N_{50}O_{36}S$) observed mass 3100.5 Da, calculated mass 3099.6 Da; CD-GCN4 ($C_{159}H_{277}N_{49}O_{69}S$) observed mass 4012.0 Da, calculated mass 4011.3 Da. Upon mixing the two peptides together, a dimer was formed through an Ad-CD inclusion complex, in this way mimicking the homodimeric transcription factor GCN4.

Binding of the GCN4 Mimic to the 1-Modified ODN's. Binding of the GCN4 transcription factor mimic to the furan modified ODNs was checked by an electrophoretic mobility shift assay. DNA duplexes for binding were prepared in 50 mM Tris buffer pH 8, with 250 mM NaCl and 2.5 mM EDTA by annealing. Increasing equivalents of peptide were added to the DNA solution with a loading buffer to obtain solutions with 6% sucrose, 100 mM Tris pH 7.6, 20 mM KCl, 10 mM $MgCl_2$, 2.5 mM EDTA, 0.167 μM of DNA, and 0, 0.42, 0.84, 1.25, and 1.67 μM peptide in lanes 1, 2, 3, 4, and 5, respectively. The binding mixtures were incubated on ice for 1 h, before 5 μL was loaded on the gel. Polyacrylamide gelelectrophoresis experiments were performed on 10 cm by 10 cm 8% acrylamide:bisacrylamide (29:1) gels, run with 0.2% TBE buffer at 150 V and 4 °C for 45 min, that had been prerun under the same conditions for 30 min. DNA was visualized by 10 min of Sybr Gold staining from Life Technologies in an Autochemi imaging system (UVP).

Standard Conditions for DPC Formation. Cross-linking reactions were performed on a 1 nmol (20 μM) DNA scale. Duplex DNA was obtained by heating 1 nmol of the 2 constituting oligonucleotides in phosphate buffer of pH 7 with 0.1 M NaCl and 0.02 M $MgCl_2$ to 95 °C for 10 min, and then allowing very slow cool down to room temperature. A total of 5 equiv of protein mimic (5 nmol) were then added and the mixture was incubated on ice for 1 h, to allow DNA−peptide complex formation. Furan oxidation was achieved by the addition of portions of NBS to the complex on ice. Since NBS will quickly oxidize the thioether bonds in Ad-GCN4 and CD-GCN4, 1 equiv of NBS should equal 11 nmol. This was added 4 times over the course of 1 h. Finally the reaction was driven to completion and the product stabilized by reduction of the formed imine to an amine with $NaCNBH_3$ (20 nmol). The reduction also restored the thioether bonds. Mass spectra for the cross-linking with CRE3 ($C_{368}H_{541}N_{113}O_{202}P_{20}S$) were obtained by using a Nano-Acquity HPLC (Waters Corporation, Milford, MA) in-line coupled to a Q-Tof Premier mass spectrometer (Waters Corporation), observed mass 10431.0 Da, calculated mass 10431.4 Da. Cross-linking yields based on HPLC were calculated by comparing the peak area from the spectra before and after addition of NBS, taking into account the extinction coefficients and corrected for sampling. For the DNA, the extinction coefficient was calculated by the nearest neighbor method, replacing the modified nucleoside with a thymidine. For the cross-linked complex, the extinction coefficient of the aromatic Aba cap (remainder of the peptide is not aromatic and does not absorb at 260 nm) was additionally taken into account. Detailed purification and characterization is described in the Supporting Information (section 2.3).

DPC under High Salt Conditions. Cross-linking was performed as described above using the CRE3 duplex, but at 1 M NaCl concentration.

DPC Attempt in Absence of GCN4 Mimic. A cross-linking experiment with CRE3 duplex in the absence of the GCN4 mimic was performed under identical conditions as described before. A volume of MilliQ water was added equal to the used volume of peptide, to ensure the same final concentration for the reaction and obtain the best comparable results.

DPC Attempt without $NaCNBH_3$ Reduction. A cross-linking experiment with CRE3 duplex was performed under identical conditions as described before, and afterward it was divided in two. Half of the sample was reduced with $NaCNBH_3$, the other half was left untreated. Both samples were stored at −20 deg for 3 weeks and analyzed together.

RESULTS AND DISCUSSION

Previously we developed several furan modified nucleosides for efficient DNA interstrand cross-linking (ICL).[28−32] For the formation of DPCs we decided to study a nucleoside (**1**) modified on the base, which positions the furan moiety away from the Watson Crick interface into the major groove where the recognition helices of proteins bind. In view of this different specific positioning of the furan moiety, it was first verified that no competitive ICL formation took place upon oxidation of the furan modified ODN in a duplex with its complementary strand (Supporting Information, section 3.1).

To apply furan-oxidation based cross-linking in a DNA−protein context, one must further take into account that oxidation or modification of methionine, tryptophan, tyrosine, histidine, and cysteine residues can occur.[34,35] However, proteins bind DNA through recognition helices, protein fragments especially rich in alanine, leucine, and arginine that have a high helix propensity[36] complemented with asparagine, glutamine, aspartate, glutamate, and arginine for specific interactions with the DNA.[37] Arginine, lysine, and histidine as charged residues further interact with the DNA backbone. These residues are highly conserved across DNA binding proteins,[38,39] illustrating their relevance. Oxidation of the earlier mentioned oxidation sensitive residues that are not conserved and do not interact with the DNA bases should thus not interfere to a major extent with DNA binding selectivity and affinity and are not expected to complicate efficient DPC formation. In the absence of proximal nucleophiles, aldehyde modified ODNs have earlier been shown to undergo self-destruction through several degradation processes within the aqueous buffer medium, further reducing the chances for collateral damage.

To study and deliver proof of concept for the possibility of cross-linking at the DNA−protein interface, a previously developed GCN4 transcription factor mimic was used as test system.[40] GCN4 is a transcription factor from yeast belonging to the basic region leucine zipper family. This transcription factor family features 2 uninterrupted α-helices that dimerize through a coiled coil and N-terminally interact with the DNA through 2 basic region peptides, or recognition helices.[41] The model system chosen here consists of these two DNA binding basic region peptides modified with either a cyclodextrin (CD) or adamantane (Ad) moiety to form a dimer through an

inclusion complex (as shown in Figure 1), which retains DNA binding selectivity and affinity of the original protein. This

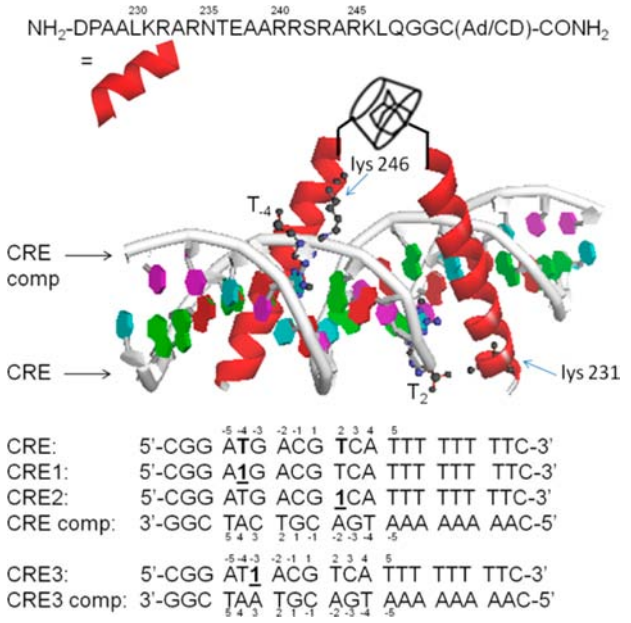

Figure 1. Illustration of the DNA binding protein mimic of GCN4 showing two lysines (lys 231 and 246) of the CD-Ad homodimer in close contact with two thymines (T) in the DNA binding site CRE:CRE comp (adapted from pbd structure 2DGC).[45]

miniaturization allows easy synthetic access, through solid phase peptide synthesis. It further allows a more straightforward analysis of conjugation products.

The synthesis of **1** and its incorporation into ODNs using phosphoramidite chemistry was described by Greco and co-workers for its fluorescent properties.[42] Wicke and co-workers obtained **1**-modified ODNs by a postsynthetic modification.[43] It was further demonstrated that **1** is amenable to enzymatic incorporation into DNA.[44] For the purpose of current investigation, furan-modified nucleoside **1** was incorporated using standard phosphoramidite protocols in the palindromic CRE DNA binding site of GCN4, consisting of two interaction sites 5′-ATGAC-3′ (numbered −5 to −1 in Figure 1) for the two DNA binding peptides. To induce minimal duplex destabilization and thus avoid loss in binding affinity, initially thymidine residues were considered for substitution by **1**. The base pair **1**-A was found to be as stable as the Watson−Crick T-A base pair, as judged from melting temperature measurements. The observed small changes in melting temperatures upon the introduction of **1** (ΔT_m(CRE1) = −1 °C, ΔT_m(CRE2) = 0 °C, ΔT_m(CRE3) = −3.1 °C) indicate minimal destabilization of the duplex by the modification. Visualization (using pdb structure 2DGC)[45] shows thymidines T-4 and T2 in proximity to the two lysines (246 and 231, respectively) of the major groove binding peptide (Figure 1). T-4 and T2 were thus replaced by **1**, in CRE1 en CRE2, respectively. From this visualization it was, however, also clear that guanosine G-3 is closest in proximity to lys 246. These moieties are involved in a water mediated amino acid−base contact.[45] Therefore, an additional sequence, CRE3, was synthesized and combined with a similarly mutated complementary strand CRE3 comp to equally obtain a **1**-A base pair. Binding of the GCN4 mimic to the furan modified CRE binding sites was verified using electrophoretic mobility shift assay (EMSA) binding studies (Figure 2).

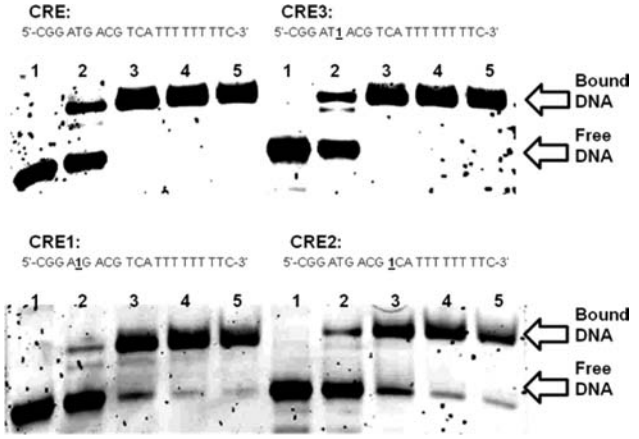

Figure 2. EMSA of the binding of the GCN4 mimic to CRE and furan modified CRE variants CRE1, CRE2, and CRE3. Lanes 1 to 5 contained increasing amount of protein mimic, respectively, 0, 0.42, 0.84, 1.25, and 1.67 μM peptide versus 0.167 μM DNA duplex in lanes 1, 2, 3, 4, and 5.

The result of the furan oxidation cross-linking reaction for the DNA−protein mimic complexes as analyzed on RP-HPLC is shown in Figure 3. The top chromatogram shows the reaction mixture containing duplex CRE:CRE comp and 5 equiv of GCN4 mimic. Under the analysis conditions, only ODNs show up in the chromatogram. After addition of 4 equiv of NBS to chemically cause furan oxidation, followed by $NaCNBH_3$ reduction to shift the equilibrium and stabilize the formed Schiff base,[46] the bottom chromatograms were obtained. In all cases, the furan modified ODN disappears, while complements remain unmodified. For CRE1 no sharp new signal can be observed, only some impurities originating from degradation of the furan modified ODN. This is in accord with the earlier observations of Ebright and co-workers, who showed a non-nucleophilic alanine residue to be closest to T−4, based on photo-cross-linking with bromouracil mutations.[47] However, for CRE2, a new small but clear signal appears. The new signal is clearly most important for CRE3, featuring closest proximity between the furan modified nucleotide and lysine. A clear relation between cross-link yield and nucleophile proximity is observed. Although in all cases an A base is located opposite modified nucleoside **1**, different product yields are obtained for the three duplexes, with a maximum yield (HPLC based) of 50% in the case of close interaction. This clearly indicates the formation of a distance dependent DPC. The complementary ODN was not consumed during the reaction in contrast to what is observed during ICL formation with other studied furan modified nucleosides (Supporting Information, Figure S3.1.1).[28−32] Furthermore, mutation of the complementary ODN to include **1**-C and even **1**-T (which has no exocyclic amine for ICL formation) base pairs, has a minor influence on the product yield (Supporting Information, section 3.2). Observed small variations are believed to be due to small changes in the binding stability and positioning of the protein mimic to the slightly different target DNA duplex. Mass analysis of the isolated product confirmed the formation of a cross-link between the modified oligonucleotide and the target peptide

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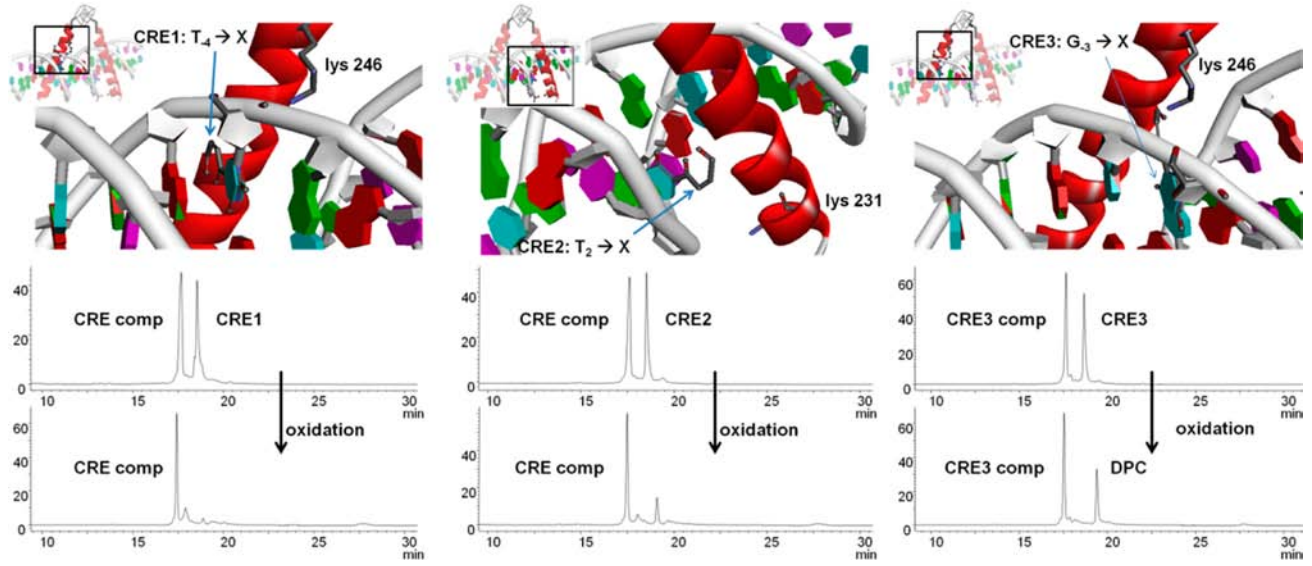

Figure 3. Cross-linking experiments. The top part of the figure illustrates the positioning of oxidized **1** in the DNA with respect to the lysine residues protruding from the protein mimic into the major groove. Images obtained from the pdb structure 2DGC using the Discovery Studio visualization software from Accelrys, the color code for the bases is as follows A = red, T = blue, G = green, and C = magenta. The bottom part shows the HPLC traces before and after furan oxidation for the duplexes of CRE1, CRE2, and CRE3 bound by the GCN4 protein mimic (which is not visible in the HPLC chromatograms).

(mass spectra available in the Supporting Information, Figures S2.3.2-S2.3.3).

To verify the necessity of proximity between the generated enal functionality and the target lysine residue, the cross-linking experiment with CRE3 was repeated at high salt concentration. While duplex stability is generally enhanced under such conditions,[48] DNA−protein complex formation will be compromised.[49] The experiment under these conditions indeed resulted in hardly any cross-link formation, owing to the destabilization of the DNA−protein mimic complex (Figure 4B). Similarly, a control experiment in the absence of protein

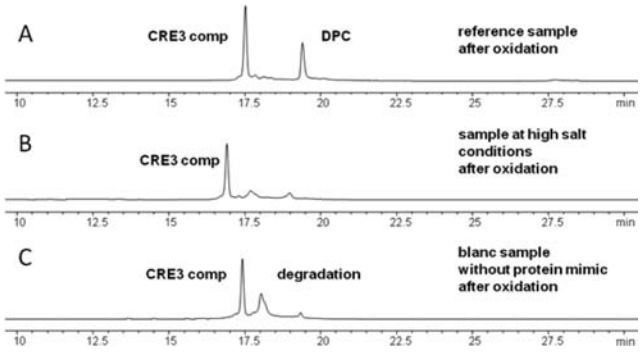

Figure 4. HPLC-traces of the cross-link reaction mixture of CRE3:CRE3 comp after NBS addition under standard conditions (A), under high salt conditions (B), and in the absence of protein mimic (C).

mimic did not yield cross-linked product (Figure 4C). Clearly, if no proximal nucleophile for selective reaction is present, random degradation of the modified ODN takes place, deactivating the cross-linking moiety. Furthermore, omitting the reduction step, the cross-linked complex was shown not to be stable (Figure 5).

Current findings can be rationalized mechanistically as illustrated in Scheme 2. The major product of furan oxidation

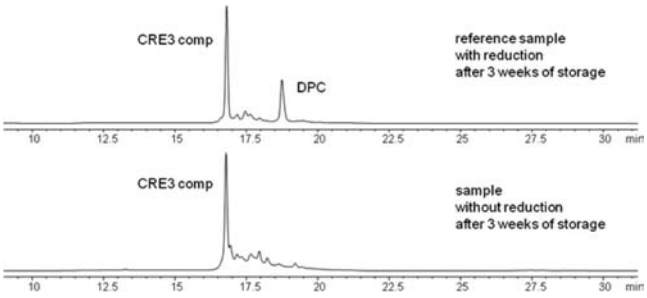

Figure 5. HPLC-traces of the cross-link reaction mixture of CRE3:CRE3 comp after NBS and NaCNBH3 addition and 3 weeks of storage (top) without NaCNBH3 addition and 3 weeks of storage (bottom).

with NBS is the highly reactive oxo-butenal, which degrades in the absence of proximal nucleophiles. The degradation mechanism is unclear and difficult to study due to the reactivity of the formed intermediate. We believe that this reactivity allows the aldehyde to react in many different ways. Besides that, nonreacted aldehyde modified DNA can be retained on RP-silica, explaining the absence of clear product formation during HPLC analysis. A furan brominated species, resulting from competitive electrophilic aromatic substitution, is only formed as a minor side product. In the presence of proximal primary amines from amino acid residues, the formed reactive aldehyde functionality generates a Schiff base that can be selectively reduced with $NaCNBH_3$ to generate a DPC.[6,11,17−20,26] Alternatively, in the case of a differently positioned furan as shown in earlier work focusing on interstrand cross-linking,[28−32] stable adducts can be formed with proximal nucleic acid bases, generating ICLs. It is clear that carefully planned differential positioning of the furan moiety on the nucleoside allows switching from ICL to DPC formation.

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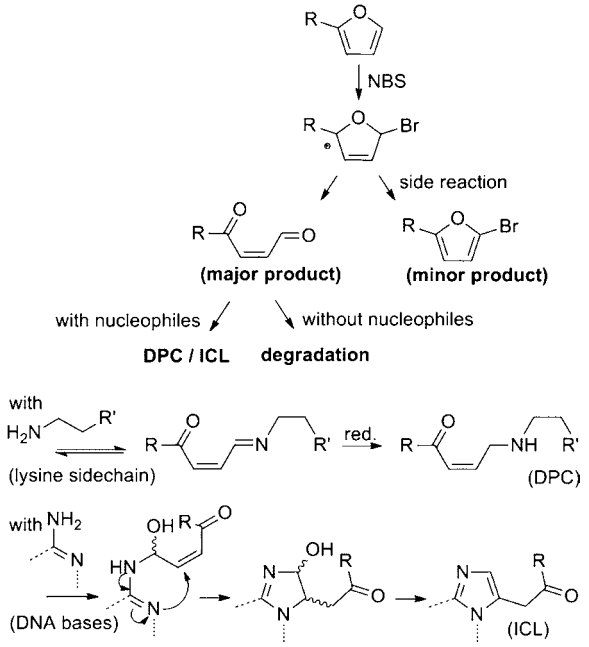
Scheme 2. Mechanism of the Furan-Oxidation Methodology for the Formation of DPCs



CONCLUSION

In conclusion, we have illustrated the use of furan as inducible DNA to protein cross-link forming moiety, inspired by the toxicity of furan. The stability and general availability of the small aromatic heterocycle allows for its easy incorporation into ODNs. Selective oxidation then generates a very reactive aldehyde that engages in a specific reaction with proximal nucleophiles or degrades in their absence. This renders this type of furan containing ODNs particularly useful as interaction or distance scanning probes for the study of DNA−protein interactions. We've shown that positioning of the furan moiety on the base, allows **1** to selectively produce DPCs in high yield. As the applicability of furan-based cross-linking has recently been clearly established in a biological context by us[31] and others[33] for nucleic acid targets, current expansion toward amino acid side chains in protein targets now considerably broadens the application area of the methodology for cross-linking and bioconjugation.

ASSOCIATED CONTENT

Supporting Information

A summary of the used ODNs names and structures, detailed procedures, additional spectral data and experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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