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Structure and function of the UVDE repair protein

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SUMMARY AND DISCUSSION

UVDE is a repair enzyme discovered for the first time in the fission yeast *Schizosaccharomyces pombe*. The initial biochemical characterization of this enzyme showed that its substrate specificity includes not only UV lesions, but also abasic sites and some nucleotide mismatches. The mechanism, however, of UVDE damage recognition and DNA cleavage was not clarified. *S. pombe* UVDE was seen to require Mn^{2+} and Mg^{2+} for its function, but the utilization of divalent metals in its cleavage reaction was empirical and without a clear vision of the nature, the number and the exact role of the metal cofactors.

UVDE homologues were found in many fungal species and in a number of bacteria such as *Bacillus subtilis* and *Thermus thermophilus*. Although the *S. pombe* UVDE was partially characterized, no information was available for the substrate specificity or the cofactor requirements its bacterial homologues.

The first glimpse at UVDE at molecular level is given by the *T. thermophilus* UVDE structure (Chapter 2). The enzyme is seen to be a single domain TIM-barrel with a wide 29 Å groove and three metals coordinating site buried deeply at bottom of the groove. Except for H224, all the residues involved in the coordination of the metal ions are fully conserved in all known UVDE homologues. Point mutations both in *T. thermophilus* UVDE (Chapter 2) and in *S. pombe* UVDE (Chapter 5) reveal the crucial importance of the divalent metals for the UVDE function, since alanine substitutions of most of the metal coordinating amino acids abrogate activity. The UVDE divalent metals superimpose on the three Zn^{2+} ions of Endo IV shedding some light on how UVDE might use them to incise DNA. A model is proposed (Chapter 2 and 5) where UVDE uses the metal ion at position 1 and probably 2 to create a hydroxide nucleophile, which attacks the phosphodiester backbone of DNA creating an unstable intermediate. The divalent metal at position 3 is likely to participate mainly in the stabilization of the intermediate step.

Although the fluorescent scan was unable to determine the exact nature of the found metals, biochemical tests suggested that they might be Mn^{2+} (Chapter 2). That Mn^{2+} is the actual UVDE cofactor is later confirmed by in detail metal substitution study (Chapter 5). Although Mn^{2+} is not the only divalent metal that UVDE can use, utilization of other metal ions is less efficient and highly dependent on the type of DNA damage. Furthermore, divalent metals are seen to have an opposite effect on different UVDE enzymes. Mg^{2+} , which positively influences the activity of *S. pombe* and *B. subtilis* UVDE by preventing their unspecific binding, completely abrogates the *T. thermophilus* incision of the CPD lesion. This unexpected effect of Mg^{2+} is clearly linked to a reduction in the stability of the protein-CPD complexes. On the 6-4PP, however, the negative influence of Mg^{2+} is neutralized by Mn^{2+} , since when both Mn^{2+} and Mg^{2+} ions are present the binding efficiency of the *T. thermophilus* UVDE is restored. These data imply that there is significant difference in the cofactors coordination when UVDE is bound to CPD or 6-4PP. Based on this finding and the outcome of the metal substitution experiments in Chapter 5 we

propose a model where the full occupation of the UVDE metal binding sites occurs only upon DNA binding and the DNA itself has an important role in the metal ions coordination.

In the crystal structure of the *T. thermophilus* UVDE an unexplained density is observed at the tip of Lys229. In Chapter 3 we give evidence that the UVDE post-translational modification is most likely carboxylation. A role for the carboxyl group is proposed in assisting the stable metal binding by donating a negative charge to the *T. thermophilus* metal coordinator His231. Lys229 is conserved in the eukaryotic UVDEs, suggesting that they might share the same modification as the *T. thermophilus* homologue. Some of the bacterial UVDEs, however, have a leucine, isoleucine, methionine, threonine or valine at the position corresponding to Lys229, thus these homologues cannot be carboxylated.

The crystal structure of UVDE gives an idea how DNA might be bound and cut by this enzyme. Initially a computer model based on the extensive structural similarities between UVDE and Endo IV suggested that UVDE most likely kinks the DNA at the site of the lesion with its GQY-loop stabilizing the kinked conformation (Chapter 2). Furthermore, in order to position the scissile phosphodiester bond close to the deeply buried metal cofactors, UVDE needs to flip the DNA damage within its active site for extrahelical repair. The initially proposed model (Chapter 2) is further elucidated in Chapter 4, where it is revealed that the initial UVDE damage recognition likely includes probing for differences of the DNA bendability. It is shown that not only a CPD, a 6-4PP or an abasic site but also a single strand nick or a gap significantly increase the binding affinity of UVDE, although not all complexes formed are catalytically active. In that regard any alteration in the DNA that increases its flexibility is a substrate for UVDE. This finding explains for the first time the previously observed broad substrate specificity of *S. pombe* UVDE. In addition, using a 2-aminopurine fluorescent probe we reveal that UVDE causes a significant destabilization in the DNA strand opposite the damage.

In the crystal structure of *T. thermophilus* UVDE Tyr105 is seen in an unusual orientation, pointing straight into the solvent. In the UVDE structural homologue Endo IV, the side-chain residue Tyr72 shows the same orientation in the absence of a DNA substrate and in the co-crystal of Endo IV with an abasic site containing DNA it projects into the kink of the DNA duplex. Based on this similarity and the fact that the alanine substitution of Tyr105 in the UVDE from *T. thermophilus* abrogates activity, we propose (Chapter 2) that this residue might be responsible for the protein-DNA interactions. The role of the tyrosine is discussed in further details in Chapter 4, where the phenotype of the *S. pombe* Y358A mutant is studied in more details. Although inactive on the abasic site lesion this mutant is highly potent in nicking the CPD and the 6-4PP lesions. We show that in the absence of Tyr358 *S. pombe* UVDE exhibits an increased catalytic activity on UV-induced lesions, but only at a lower pH of 6.5. At physiological conditions (pH 7.5) the mutant protein completely loses its catalytic activity, although it can still bind to the DNA. Based on these findings we propose that in addition to stabilizing the bend in the DNA the hydrophobic side chain of Tyr358 has a role in shielding the active site from solvent exposure.

Although originally claimed that UVDE is an alternative repair pathway for UV lesions, abasic sites, mismatches and oxidative damages we clearly demonstrated that this is not true for all UVDE homologues. We show in Chapter 4 that *S. pombe* UVDE incises DNA containing a single-strand nick or gap, but that the enzymatic activity on these substrates as well as on abasic sites strongly depends on the presence of a neighbouring pyrimidine residue. This indicates that although UVDE may have been derived from an ancestral AP endonuclease its major substrates are UV lesions and not an AP site. Furthermore, the bacterial UVDE homologues do not share the broad substrate specificity of the eukaryotic *S. pombe* protein and the enzyme from *T. thermophilus* functions as a UV damage specific nuclease.