THE REPAIR ENZYME URACIL-DNA-GLYCOSYLASE: STUDY OF THE MECHANISM OF FUNCTIONING USING MODIFIED ANALOGUES OF DNA

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The pecularities of enzyme-substrate recognition for uracil-DNA-glycosylase (UDG) from human placenta by using synthetic DNA were discussed. Our biochemical results were compared with X-ray analysis data. The DNA structural elements responsible for UDG binding and catalysis were identified. The fast and efficient method for testing activity of UDG was described.

Uracil-DNA-glycosylase (UDG) is the first enzyme in the base excision repair pathway for removal of uracil in DNA. The disturbance of reparation system results in occurrence of a number of diseases, including various kinds of a cancer. Uracil (Ura) in DNA may result from incorporation of dUMP instead of dTMP or from spontaneous deamination of cytosine in DNA. The majority of organisms delete the erroneous uracil bases from DNA by UDG, which cleaves N-glycosylic bond between this base and the deoxyribose residue to give an apyrimidinic site [1].

UDG sequences from different sources (human placenta, *E.coli*, *Streptococcus pneumonia*, different herpes viruses etc) are closely related. They are small single-polypeptide chain enzymes with no cofactor requirement [2].

The aim of the present work is: (i) to summarized our biochemical data which was obtained for UDG from human placenta by using synthetic substrate analogs and characterize UDG-DNA interaction in solution; (ii) to compare our results with X-ray data for complex of mutant non-active UDG with 2′-deoxyuridine (dU)-containing DNA duplex [3].

Results and Discussion

Express method of testing activity of the uracil-DNA-glycosylase. UDG was isolated from human placenta as discribed in [4] and was kindly granted by prof. G. A. Nevinsky. The rapid methods of the analysis of the UDG activity, particulary at the intermediate stages of the UDG isolation, are absent.

We suggest an express method for testing activity of UDG [5]. In this method, 5'-^{32}P-labelled dU-containing oligodeoxyribonucleotide (oligo) that was irreversibly attached to polystyrene matrix (Tenta Gel S–NH_2) was used as a substrate for UDG. Cleaved at the apyrimidinic site, the ^{32}P-labelled fragment of oligonucleotide passes from the solid support to solution. Ammonia hydrolysis of the apyrimidinic site after the reaction with UDG instead of piperidine results in the decreasing of the reaction time. The enzyme activity was registrated as a ratio of the solution/solid support radioactivity (Fig. 1).

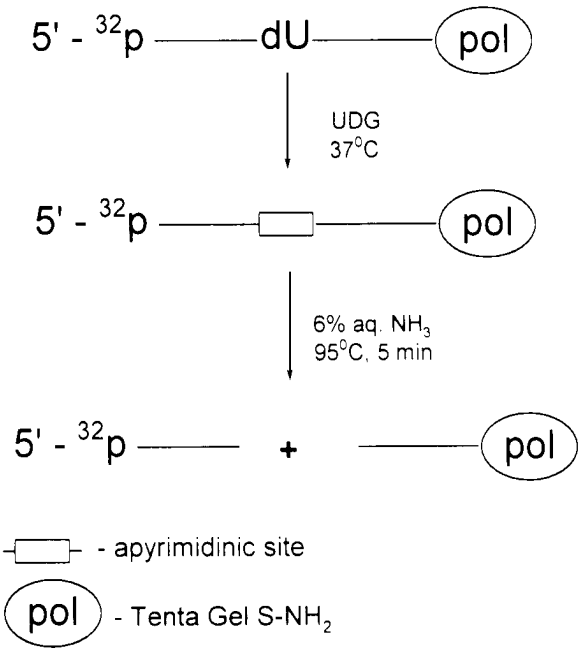

Fig. 1. Scheme of the testing activity of the uracil-DNA-glycosylase.

Characterisation of UDG-DNA interaction based on biochemical data. The molecular bases of the mechanisms of uracil recognition and elimination from DNA catalyzed by UDG were studied using specially modified DNA analogues [6, 7].

UDG interaction with single- and double-stranded oligos. The rate of uracil removal from oligo I, duplex II, hairpin III and dumbbell IV by UDG were compared (Table 1). UDG reveals 2-fold preference for single-stranded oligo I over duplex II and 1.5–2.5-fold preference for duplex II over hairpin III which is 30 °C more stable. The most stable dumbbell IV is very poor substrate for UDG. These data indicate that UDG functioning is more effective when double helix is less stable. The local DNA unwinding is possible on the first step of UDG action.

The role of heterocyclic bases and phosphate groups in UDG-DNA interactions. We have demonstrated recently

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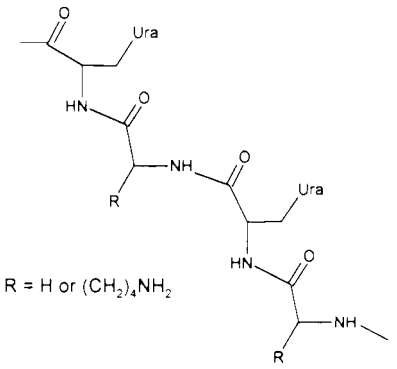
Table 1

Interaction of UDG with single- and double-stranded oligos

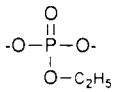
	DNA duplex	Rel. v_0	Melt. point, °C (H_2O)*
I	**5′ GCCAACCUGGCTCTp**	2.30	—
II	5′ **pGCCAACCUGGCTCT** 3′ **CGGTTGGACCGAGACA(A)$_3$TG**	1.00	27
III	**(A)$_3$ ACGCCAACCUGGCTCTp** **TGCGGTTGGACCGAGACA(A)$_3$TGp**	0.60	66
IV	**(A)$_3$ ACGCCAACCUGGCTCTppGT (A)$_3$** **TGCGGTTGGACCGAGA—CA**	0.02	82

* Synthesis and determination of the thermal stability of oligos II–IV was performed by Kuznetsova et al. [8].

that there is no considerable contribution of heterocyclic bases to UDG-substrate recognition [7]. Thus, the affinity of oligomer d[(pX)$_9$pT] with nine abasic sites instead of heterocyclic bases (X = (2R,3S)-2-oxymethyltetrahydrofuranol-3) is comparable with that for d(pC)$_9$. To elucidate the effect of DNA phosphate groups in UDG-DNA interaction two types of DNA analogs, in which the negative charges of backbone were eliminated, were probed as competitive inhibitors of UDG [7]. All internucleotide phosphate groups were ethylated in oligomer d[(TpEt)$_{14}$T]. The ethylation of phosphate groups caused at least 4-fold decrease of UDG binding to this oligo. 21-membered oligonucleotidepeptide (ONP) contains the Ura residues but its sugar–phosphate backbone was substituted by noncharged peptide chain.



ONP



pEt

Despite on the presence of Ura residues ONP also demonstrated poor binding to UDG.

Thus, the UDG interaction with some internucleotide phosphate groups is necessary for enzyme–DNA recognition.

The role of the substituent at position C5 of uracil in UDG–DNA interaction. Fluorine (fl) or bromine (br) atoms or a CH_3 group were introduced at position C5 of dU residue in oligos (Table 2). UDG binding to oligos V-VIII and the removal of uracil or its analogues were investigated [6]. The introduction of a substituent at C5 position of dU does not influence on UDG binding with the substrate analogue. Thus, UDG does not discriminate Ura from other heterocyclic bases during the binding step. However, UDG does not remove 5-bromouracil and thymine from DNA, fluorouracil also is almost not excised (Table 2). The hydrogen atom at C5 of uracil obviously is involved in the interaction with UDG at the catalytic step.

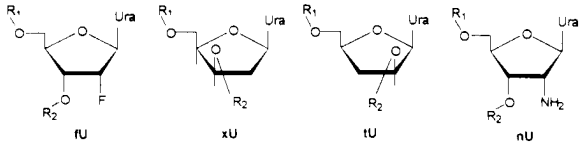
Table 2

Interaction of UDG with synthetic substrate analogs

	Oligo* (5′ → 3′)	Rel. affinity to DNA**	% of uracil removal
V	... CC**U**GG...	1.00	96
VI	... CC**T**GG...	1.10	0
VII	... CC**U**^{fl}GG...	2.00	13
VIII	... CC**U**^{br}GG...	0.40	0
IX	... CC**f**UGG...	0.01	0
X	... CC**x**UGG...	1.60	6
XI	... CC**t**UGG...	0.80	1
XII	... CC**n**UGG...	2.50	0

* Only central pentanucleotide fragments of 14-membered oligos are presented.
** Relative UDG affinity was estimated as ratio of K_M for substrate V to K_I for modified oligos.

Interaction of UDG with substrate analogues containing unusual sugar moieties. Introduction of single 2′-fluoro-2′-deoxyuridine (fU), 1-(β-D-2′-deoxy-*threo*-pentofuranosil)uracil (xU), 1-(β-D-3′-deoxy-*threo*-pentofuranosil)uracil (tU) or 2′-amino-2′-deoxyuridine (nU) into the oligo 5′-d(GCCAACCUGGCTCT)-3′ instead of dU causes the local conformational perturbations of DNA substrates.



The sugar puckering in fU residue is 3′-*endo* instead of 2′-*endo* conformation in dU [9]. The fU-containing oligo IX is recognised by UDG 100–200-fold less than dU-substrate and the Ura excision does not take place [6]

(Table 2). Unusual configuration of hydroxyls in xU and tU residues following by disturbance of internucleotide phosphate group orientation [6] and addition of bulky NH_2 group in 2′-position of sugar [7] do not influence on the UDG binding to oligos X–XII but inhibit the Ura removal (Table 2).

Thus, conformation of the ribose may be determined factor for DNA binding to UDG but not for uracil excision. The 3′-*endo* conformation of the sugar moiety prevents UDG interaction with DNA. The contact or proximity of UDG with C2′ atom of dU is possible.

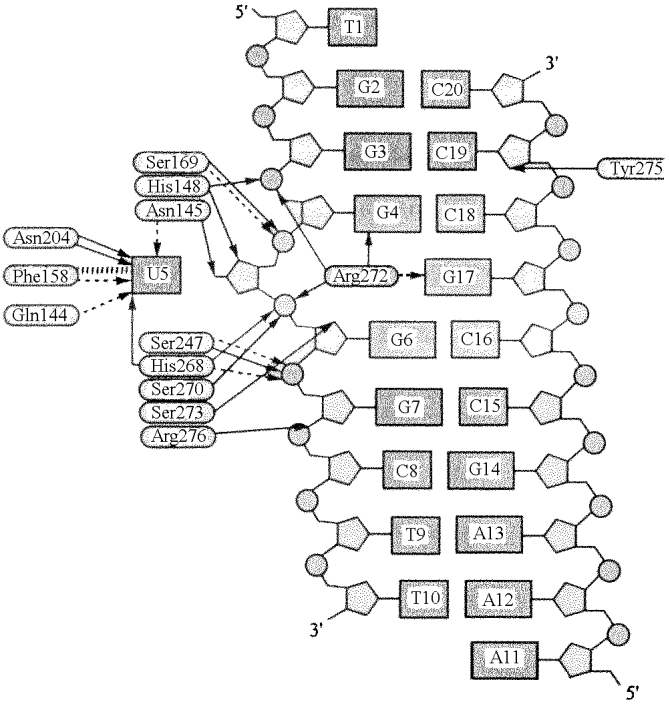


Fig. 2. Interactions of UDG from human placenta with DNA [3]. Hydrogen-bond interactions with DNA are shown for amino-acid side chains (solid lines) and backbone atoms (dashed lines). Stacking interaction is shown for Phe158 to uracil (thick broken line).

X-ray analysis data. Recently X-ray data for complex of mutant non-active UDG with dU-containing DNA duplex was obtained (Fig. 2) [3]. Following conclusions about DNA-UDG interaction could be done based on these results. **(i)** Protein-DNA interactions are mainly concentrated along the sugar-phosphate backbone of uracil containing strand. **(ii)** The local DNA unwinding takes place followed by uracil flipping out. **(iii)** The flipped-out uracil binds within the UDG specificity pocket. The uracil stacks with Phe158 and forms hydrogen bonds via its O4, N3 and O2 atoms to five UDG-residues: NH-group of Phe158, side chain of Asn204, the backbone atoms of residues Gln144 and Asp145 and imidazole of His268. The selecting against thymine occurs due to Tyr147 packing near uracil C5-position. **(iv)** The deoxyribose forms hydrogen bonds from O4′ and O1′ to His 148 and Asp145 respectively. The substituents at 2′-position of deoxyribose and 3′-endo puckering block His268 movement and prevent the hydrolysis of substrate by UDG. **(v)** The 5′-phosphate group of dU forms two hydrogen bonds with Ser169 during the catalytic step, the 3′-phosphate group of dU forms hydrogen bonds with Ser270 and His268.

Thus, our biochemical results are in good agreement with X-ray data. The basic features of UDG-DNA interaction in solution and crystals state are: (1) UDG binds with uracil-containing strand of DNA; (2) UDG interaction with some internucleotide phosphate groups is necessary for enzyme-substrate recognition; (3) UDG does not discriminate Ura from other heterocyclic bases during the binding step; (4) 3′-endo conformation of sugar prevents the UDG-DNA interaction; (5) the local DNA unwinding takes place during catalytic step; (6) C5 and C2′ positions of dU are involved in formation of active enzyme-substrate complex.

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