Selective Inhibition of Herpes Simplex Virus Type-1 Uracil-DNA Glycosylase by Designed Substrate Analogs

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters.

Citation
Sekino, Yukiko, Steven D. Bruner, and Gregory L. Verdine. 2000. “Selective Inhibition of Herpes Simplex Virus Type-1 Uracil-DNA Glycosylase by Designed Substrate Analogs.” Journal of Biological Chemistry 275 (47): 36506–8. https://doi.org/10.1074/jbc.C000585200.

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:41511292

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Selective Inhibition of Herpes Simplex Virus Type-1 Uracil-DNA Glycosylase by Designed Substrate Analogs*

Received for publication, August 28, 2000
Published, JBC Papers in Press, August 31, 2000,
DOI 10.1074/jbc.C000585200

Yukiko Sekino, Steven D. Bruner, and Gregory L. Verdine†‡
From the Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138

UDGs are highly conserved class of DNA repair enzymes that initiate excision repair of uracil by hydrolyzing the N-glycosidic bond (Fig. 1a). Uracil in DNA arises from the misincorporation of deoxyuridine triphosphate (dUTP) during replication and from the hydrolytic deamination of cytosine (1, 2). Cytosine deamination generates highly mutagenic G:U mismatches that lead to G:C to A:T transition mutations. Characterized by a high substrate specificity, UDGs can distinguish between such structurally similar bases as uracil and thymine. Unlike other DNA glycosylases, UDGs remove uracil from both single-stranded and double-stranded DNA, often with higher efficiency for the single-stranded substrates (1, 3). DNA glycosylases accomplish the formidable task of scanning a large excess of normal DNA bases, recognizing and then catalyzing the excision of damaged bases. A molecular-level description of how DNA glycosylases achieve recognition and repair requires the formation of stable protein-substrate complexes that can be studied by x-ray crystallography or NMR. Such studies are not ordinarily possible because of the transient nature of the enzyme-substrate interaction. One way to circumvent this problem involves mutating the enzyme so as to obtain a catalytically inactive form that retains the ability to recognize its substrate (4, 5). An alternative approach is to effect “mutate” the substrate by preparing synthetic analogs that bind the enzyme specifically but cannot be processed by it (6–8). Both approaches have proven useful in structural and biochemical analysis of base excision DNA repair (4–12). Powerful features of the substrate modification approach are that it requires no prior knowledge of the enzyme active site and that it can be structurally very conservative, entailing changes of as little as a single atom.

UDGs have an unusually broad phylogenetic distribution, being present in organisms from the simplest free-standing bacterium (13) to plants (14) to man (15). Furthermore, UDGs have been identified in the genomes of herpesvirus and poxvirus. HSV-1 UDG is dispensable for HSV-1 replication in cell culture, presumably because the cellular UDG can supplant the activity of the viral enzyme (16, 17). However, an intact HSV-1 UDG gene appears to be necessary for efficient viral replication and reactivation in the murine nervous system (18). The difference in the efficiency of viral replication and reactivation between the in vitro and in vivo studies has been attributed to the lack of detectable endogenous UDG expression in neurons, which are terminally differentiated (19). Studies of HSV-1 in neural systems are relevant because HSV-1 establishes latency in the neuronal ganglia (20). Despite these results, the role of UDG in herpesvirus replication and reactivation remains unclear. For example, varicella-zoster virus (VZV), another member of the herpesvirus family, has been shown to replicate in the absence of detectable endogenous or host UDG activity (16). Human cytomegalovirus (CMV), another herpesvirus, is unlike HSV-1 or VZV in that the disruption of its UDG gene results in a longer replication cycle with delayed DNA synthesis in tissue culture (21). Potent and highly specific inhibitors of viral UDGs could serve as valuable tools to elucidate the in vivo role of the enzyme in herpesvirus replication and virulence. The availability of such molecules could provide the impetus to pursue HSV-1 UDG as a novel target for antiviral therapy (22).

Despite the potential therapeutic interest in HSV-1 UDGs, few synthetic inhibitors of UDGs have been reported. The most potent non-protein UDG inhibitors thus far reported have IC50 values in the micromolar range (23, 24). Here, we report the design, synthesis, and evaluation of two inhibitors of UDGs. Our results demonstrate that these two oligonucleotide-based inhibitors bind and inhibit HSV-1 UDG while having little effect on the human enzyme.

EXPERIMENTAL PROCEDURES

Enzymes—HSV-1 and human UDG (UDG84) were generous gifts from Prof. Laurence H. Pearl (Department of Biochemistry and Molecular Biology, University College, London) and Prof. Hans Krokan (UNI-GEN Center for Molecular Biology, The Norwegian University of Sci-
ence and Technology, respectively.

Oligonucleotide Substrates/Competitors—All oligonucleotides were synthesized by standard β-cyanoethyl solid phase chemistry on a 1 μmol scale using an ABI model 392 DNA synthesizer and purified by denaturing polyacrylamide gel electrophoresis. End-radiolabeling reactions were performed with T4 polynucleotide kinase (New England Bio-labs) and α32P[ATP (PerkinElmer Life Sciences). Duplexes were prepared by annealing with 10-fold excess of complementary strand. The radiola-beled strand of each duplex is the top strand, as shown in Sequence 1. The phosphoramidite of 2'-β-fluoro-2'-deoxyuridine βFdUrd) was synthesized according to published procedures (25). The phosphoramidite of farnum honourium (fhUrd) was synthesized by an adaptation of a published procedure (26).

Electrophoretic Mobility Shift Assays (EMSA)—The standard binding reaction mixture (20 μl) contained varying amounts of protein and 0.4 fmol of32P-labeled oligonucleotides in 100 μM NaCl, 0.5 mM EDTA, 0.5 mM dithiothreitol, 50 mM Tris, pH 7.4. After 30 min of incubation at room temperature, the samples were resolved on a 10% native polyacrylamide gel in 0.5X Tris-borate-EDTA running buffer. The thermody-namic dissociation constants (Kd = [protein][DNA]/[protein-DNA com-plex]) were measured as the concentration of the protein at which half of the target DNA is bound, under conditions in which [DNA] ≪ [protein]. Data from at least three titration gels were averaged to obtain the reported Kd values, which have standard errors of ±50%. Example gels and binding data used to determine the experimental equilibrium constants are available as supplemental information.

Cleavage Inhibition Assays—The standard reaction mixture (20 μl) contained 20 fmol of32P-labeled UDG duplex oligonucleotides, varying dilutions of enzyme and 1 pmol of competitor oligonucleotide duplex. The reaction buffers were as follows: human UDG (60 μM NaCl, 20 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, pH 8); HSV-1 UDG (20 mM Tris-HCl, 10 mM EDTA, pH 8). The reactions were incubated for 30 min at 37°C for HSV-1 UDG and 10 min at 30°C for human UDG and were stopped by the addition of 10 μl of formamide loading dye and 5 μl of 2 M piperidine. The sample mixtures were then boiled for 30 min prior to resolving on a 20% polyacrylamide gel.

RESULTS AND DISCUSSION

Design and Synthesis of UDG Inhibitors—Like all DNA glycosylases, UDG processes its substrate through a transition state involving (SN1) character, whereas positive charge is accumulated on the sugar ring, especially at C-1 and O-1 (27–29). Inhibitors have been designed either to mimic this transition state or to destabilize it electronically. The latter principle was employed in this study. Namely, analog 1 (2'-β-fluoro-2'-deoxyuridine, βFdUrd) (Fig. 1c) possesses a 2'-fluoro substituent, which destabilizes the transition state by withdrawing electron density from the already electron-deficient C-1 center. Analog 2 (fhUrd) (Fig. 1c) interposes a CH2 unit between the base and its sugar, thereby depriving the transition state of assistance from the lone pair electrons on O-1. The increased distance from C-1 to the base in 2 may resemble the lengthening of the glycosidic bond that occurs during displacement of the base (Fig. 1b). Both analogs possess an attached uracil base, which UDG is expected to recognize specifically. Indeed, analog 1 has previously been shown to bind and inhibit the human G/T glycosylase at subnanomolar concentrations (12).

The βFdUrd and fhUrd nucleoside analogs were synthesized and incorporated into the middle of 25mer oligonucleotides using standard solid phase phosphoramidite chemistry. For

2 Y. Sekino, S. D. Bruner, and G. L. Verdine, unpublished results.

The action of UDG and designed inhibitors of UDG. a. UDG catalyzes the hydrolysis of the N-glycosidic bond between uracil and the deoxyribose backbone. b. The proposed transition state for glycosidic bond cleavage by UDG. c. Designed inhibitors of UDG.

double-stranded inhibitors, inhibitor oligonucleotides were annealed with complementary oligonucleotides containing a G opposite the analog.

Binding Interactions between HSV-1 and Human UDGs and the Inhibitors—The binding affinities of the HSV-1 and human UDGs for the designed inhibitors were evaluated by EMSA (Fig. 2). HSV-1 UDG binds the double-stranded (ds) βFU inhibitor tightly with an apparent Kd of 4 nM. Under the same experimental conditions, the dsβFU inhibitor fails to form a specific gel shift complex with human UDG. A 25mer containing inhibitor 2 was tested in the same way using EMSA and was found similarly to bind with high specificity to only the viral UDG, albeit with lower affinity (Kd = 160 nM).

UDGs are known to excise uracil from both single-stranded (ss) and dsDNA. We therefore tested the binding of HSV-1 and human UDGs to inhibitors 1 and 2 in ssDNA. Although HSV-1 UDG does not bind detectably to the ssfhUrd inhibitor, HSV-1 UDG retained nanomolar affinity for the ssβFdUrd inhibitor (Kd = 47 nM). Neither uracil analog in ssDNA bound the human UDG even at concentrations as high as 1 μM. Thus, the βFdUrd inhibitor is exquisitely specific for the viral over the human UDG, irrespective of whether the DNA is single- or double-stranded.

Specific Inhibition of HSV-1 UDG Activity by βFdUrd and fhUrd Inhibitors—To determine whether binding of the analogs to UDG results in inhibition of catalytic activity, we analyzed the ability of the analogs to interfere with cleavage of a natural substrate by UDG. In this assay, a 5'-32P-labeled uracil-containing substrate is incubated with UDG in the presence or absence of unlabeled competitor analog. Processing of the natural substrate creates an abasic site, which undergoes strand cleavage to generate shortened oligonucleotide products upon treatment with aqueous piperidine. As shown in Fig. 3a, a 50-fold excess of dsβFU gives nearly complete inhibition (~90%) of HSV-1 UDG activity (lane 4). The ssfhUrd oligonucleotide also inhibited HSV-1 UDG activity strongly (lane 7). Under the conditions of these assays, the fhUrd analog inhibited cleavage by ~40% in dsDNA (lane 5) but not to any significant extent in ssDNA (lane 8). By contrast, neither analog caused pronounced inhibition of human UDG; although βFU in both ssDNA and dsDNA showed evidence of modest inhibition (~25% relative to nonspecific DNA, Fig. 3b). Overall, the trends seen in cleavage inhibition assays closely parallel those in binding assays, consistent with the notion that these analogs target the enzyme active site.
notably of human, *Escherichia coli*, and HSV-1 UDGs (4, 30–34), have elucidated certain key features of uracil excision repair, tight complexes of a native UDG enzyme with an intact DNA substrate or substrate analog have not previously been available for structural studies. The precise mechanism by which these enzymes scan the genome to locate and excise uracil residues remains unknown. The present inhibitors allow the formation of such stable complexes and are thus expected to shed further insight into the process of substrate recognition and catalysis by UDGs.

Viral infections represent a major human health concern with few therapeutic options available. The fact that many members of the poxvirus and herpesvirus families carry a functional UDG in their limited genome and that other viruses, including the human immunodeficiency virus type 1, actively package host UDG protein into viral particles suggests a potentially important functional role for this enzyme (35). However, the precise function of UDGs in viral infection remains unclear. Highly selective and specific inhibitors described in this communication provide new reagents to probe the *in vivo* role of viral UDGs. Although oligonucleotides often suffer from poor cell permeability, there is reason to be optimistic in the case of these inhibitors, as oligonucleotides containing as few as 7 nucleotides inhibit HSV-1 UDG in vitro.²

**Acknowledgments**—We thank Prof. Lawrence H. Pearl and Prof. Hans E. Krokan for their generous gifts of HSV-1 and human UDGs, and Orlando Schärer for synthesis of the βFU oligonucleotides. We thank Xiaoyan Zhang, Karl Haushaulter, and Derek Norman for critical readings of this manuscript, and members of the Verdine laboratory for helpful discussions.

**REFERENCES**

1. Mosbaugh, D. W., and Bennett, S. E. (1994) *Proc. Nucleic Acid Res. Mol. Biol.* 21, 315–370
2. Lindahl, T. (1993) *Nature* 362, 709–715
3. Eftedal, I., Guddal, P. H., Slupphaug, G., Volden, G., and Krokan, H. E. (1993) *Nucleic Acid Res.* 21, 2095–2101
4. Slupphaug, G., Mol, C. D., Kavli, B., Arvai, A. S., Krokan, H. E., and Tainer, J. A. (1996) *Nature* 384, 87–92
5. Vassylyev, D. G., Kashigashi, T., Mikami, Y., Aritoyshi, I., Isaiwa, S., Ohtsuka, E., and Morikawa, K. (1995) *Cell* 83, 773–782
6. Lauer, Y., Schärer, O. D., Samson, L., Verdine, G. L., and Ellenberg, T. (1998) *Cell* 95, 249–258
7. Schärer, O. D., Deng, L., and Verdine, G. L. (1997) *Curr. Opin. Chem. Biol.* 1, 526–531
8. Barrett, T. E., Schärer, O. D., Savva, R., Brown, T., Jiricny, J., Verdine, G. L., and Paul, L. H. (1999) *EMBO J.* 18, 6599–6609
9. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) *Cell Biol.* 4, 683–702
10. Manuel, R. C., Latham, K. A., Dodson, M. L., and Lloyd, R. S. (1995) *J. Biol. Chem.* 270, 2652–2661
11. Labahn, J., Schärer, O. D., Long, A., Ezaz-Nikpay, K., Verdine, G. L., and Ellenberg, T. E. (1996) *Cell* 86, 821–829
12. Schärer, O. D., Kawate, T., Gallinari, P., Jiricny, J., and Verdine, G. L. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 4878–4883
13. Cole, S. T., Bruschi, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eglmeier, K., Gas, S., Barry, C. E., Tekaia, F., Badeck, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R., Devlin, R., Feltwell, T., Genta, S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Barrett, B. G., et al. (1998) *Nature* 393, 537–544
14. Blaisdell, P., and Warner, H. (1983) *J. Biol. Chem.* 258, 1603–1609
15. Haug, T., Skorpen, F., Kvaale, K., Eftedal, I., Lund, H., and Krokan, H. E. (1996) *Genomics* 36, 408–416
16. Reddy, S. M., Williams, M., and Cohen, J. L. (1998) *Virology* 251, 393–401
17. Mullaney, J., Moss, H. W., and McGeoch, D. J. (1989) *Cell* 54, 449–454
18. Pyles, R. B., and Thompson, R. L. (1994) *J. Virol.* 68, 4963–4972
19. Focher, F., Marazzotto, P., Verri, A., Hubsher, U., and Spadari, S. (1990) *Mutat. Res.* 237, 65–73
20. Chadbarg, M. D., and Kelly, T. J. (1998) *Annu. Rev. Biochem.* 67, 711–717
21. Fuchard, M. N., Duke, G. M., and Mocarski, E. S. (1996) *J. Virol.* 70, 3018–3025
22. Argnani, R., Focher, F., Zucchin, S., Verri, A., Wright, G. E., Spadari, S., and Manservigi, R. (1995) *Virology* 211, 307–311
23. Focher, F., Verri, A., Spadari, S., Manservigi, R., Gambino, J., and Wright, G. E. (1993) *Biochemistry* 32, 1153–1162
24. Argnani, R., Focher, F., Zucchin, S., Verri, A., Wright, G. E., Spadari, S., and Manservigi, R. (1995) *Virology* 211, 307–311
25. Kline, P. C., and Schramm, V. L. (1995) *Biochemistry* 34, 1513–1602
26. Erion, M. D., Stoeckler, J. D., Guida, W. C., Walter, R. L., and Ealick, S. E. (1998) *Science* 280, 2344–2350
27. Horenstein, B. A., Parkin, D. W., Estupinan, B., and Schramm, V. (1991) *Biochemistry* 30, 10778–10795
28. Kline, P. C., and Schramm, V. L. (1995) *Biochemistry* 34, 1513–1602
29. Argnani, R., Focher, F., Zucchin, S., Verri, A., Wright, G. E., Spadari, S., and Manservigi, R. (1995) *Biochemistry* 34, 1513–1602
30. Mol, C. D., Arvai, A. S., Sanderson, R. J., Slupphaug, G., Kavli, B., Krokan, H. E., Mosbaugh, D. W., and Tainer, J. A. (1995) *J. Virol.* 70, 701–708
31. Mol, C. D., Arvai, A. S., Slupphaug, G., Kavli, B., Alseth, I., Krokan, H. E., and Tainer, J. A. (1995) *Cell* 80, 869–878
32. Savva, R., McBeeley-Heck, K., Brown, T., and Pearl, L. (1995) *Nature* 373, 487–493
33. Savva, R., and Pearl, L. H. (1995) *Nat. Struct. Biol.* 2, 752–757
34. Xing, G., Tordova, M., Jagadesso, J., Dehhat, A. C., Stivers, J., and Gilliland, G. L. (1999) *Proteins* 35, 13–24
35. Willett, K. E., Rey, F., Agostini, I., Navarro, J. M., Baudat, Y., Vigne, R., and Sire, J. (1999) *J. Virol.* 73, 1682–1688

**FIG. 2.** EMSA assay comparing the binding affinities of HSV-1 and human UDG for dsβFUrd (a) and dsfhUrd (b). Concentration of 32P-labeled oligonucleotides = 0.4 nm.

**FIG. 3.** Cleavage inhibition of HSV-1 UDG (a) and human UDG (b) using both single-stranded and double-stranded βFUrd and fhUrd analogs. Concentration of 32P-labeled uracil-containing substrate = 1 nm; concentration of competitor = 50 nm. *ns*, nonspecific competitor containing C in the place of U.
Selective Inhibition of Herpes Simplex Virus Type-1 Uracil-DNA Glycosylase by Designed Substrate Analogs
Yukiko Sekino, Steven D. Bruner and Gregory L. Verdine

J. Biol. Chem. 2000, 275:36506-36508.
doi: 10.1074/jbc.C000585200 originally published online August 31, 2000

Access the most updated version of this article at doi: 10.1074/jbc.C000585200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2000/11/28/275.47.36506.DC1

This article cites 35 references, 8 of which can be accessed free at
http://www.jbc.org/content/275/47/36506.full.html#ref-list-1