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U-U and T-T cyclobutane dimers have different mutational properties

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ABSTRACT

We have examined the mutagenic properties in *E.coli* of single stranded vectors containing a uniquely placed *cis-syn* or *trans-syn* uracil – uracil cyclobutane dimer in the sequence 5′ GCAAGUUGGAG 3′, and compared these with the properties of the corresponding T-T dimers in the same sequence context. The frequencies with which U-U and T-T photoproducts were bypassed were similar in SOS induced cells, and each induced similar frequencies of mutations. However, although both U-U and T-T *cis-syn* dimers showed a preference for misincorporation in about 5 – 7% of the replication products, with T or G being incorporated in place of A, the ratios of these events differed, being >4:1 for T-T *cis-syn*, but only 2:1 for U-U *cis-syn*. A shift towards G insertion opposite dimerized uracil was also found with the *trans-syn* dimers, but the difference was greater; T and G were misincorporated opposite the U-U *trans-syn* dimer in a ratio of 1:2, compared with 4:1 for its T-T counterpart. In addition, the U-U dimer induced only nucleotide substitutions, unlike the T-T photoproduct which induced single nucleotide deletions as well as substitutions. We conclude that even relatively minor differences in photoproduct structure, such as the presence of a methyl group at C-5, can alter mutational properties, and that such properties cannot depend only on the attributes of the DNA polymerase. Neither the efficiency of bypass, the error frequency nor the mutation spectrum of either U-U isomer is influenced by DNA uracil glycosylase. *In vitro*, the U-U *cis-syn* dimer is a substrate for DNA photolyase, but not for the glycosylase.

INTRODUCTION

Determination of the mutagenic properties of individual ultraviolet light-induced DNA photoproducts *in vivo* provides an opportunity for investigating two important questions: (i) why are these photoproducts mutagenic and more specifically, what is the relationship between the chemical structure of the mutagenic lesion and its mutagenic activity? and (ii) what contribution does the photoproduct make towards the spectrum of UV-induced mutations? The study of uracil-containing cyclobutane dimers is of particular value in both of these contexts. Regarding the first issue, the importance of the chemical structure of the photoproduct in determining its mutagenic potential can be examined by comparing the properties of the closely similar U-U and T-T cyclobutane dimers. It has been argued that cyclobutane dimers are non-instructive or non-pairing lesions, and that nucleotide insertion opposite them is determined by the properties of the DNA polymerase (1). DNA polymerases are thought to preferentially insert purines, particularly adenine, at such sites and cyclobutane dimers were therefore believed to be subject to an 'A rule' (2). Although a number of observations question the validity of such an interpretation (3–5), no consensus appears to have been reached on the issue, and other recent experimental results have been interpreted according to the 'A rule' (6–7). A comparison between the mutagenic properties of uracil and thymine containing dimers therefore provides an opportunity to re-examine this issue; the importance of the chemical structure of the photoproducts, rather than just DNA polymerase properties, would be indicated if these dimers exhibit different mutational properties.

Determination of the mutagenic properties of uracil-containing dimers is also important with respect to a second issue, namely the relative contributions made by different photoproducts to the spectrum of UV-induced mutations. A number of apparently conflicting observations might be resolved if a significant number of UV-induced mutations resulted (at least in the more slowly replicated genomes) from the deamination of cytosine to uracil in cyclobutane dimers, followed by accurate replication past the lesion. Though estimates of the rate of deamination vary considerably (8–10), it is much enhanced following dimer formation and the possible importance of this event for UV mutagenesis has been pointed out (8–9). Apparently conflicting findings include the observations that whereas the non-photoreversible pyrimidine (6–4) pyrimidone adducts appear to be much more mutagenic than cyclobutane dimers (5,11), UV-induced mutations are highly photoreversible, even when SOS induction is maintained (12). Similarly, although T-T (6–4) adducts are the most mutagenic lesion studied (5), mutations often occur principally at C-C or T-C bipyrimidine sites, even in excision deficient strains (13,14). Finally, at least two patterns

of mutagenesis can be found in *E.coli*, one characteristic of the rapidly replicating M13 and lytic λ phage, in which UV-induced mutations typically occur at T-T and T-C sites, and another characteristic of the more slowly replicating F′ plasmid, in which C-C and T-C target sites predominate (5). These observations might be reconciled if a fraction of the mutations induced by UV arose by deamination of cytosine, with the proportion depending on the amount of time elapsing before replication. To explain the observations in this way requires, however, that: (i) bypass of dimers results in the insertion of A, and not G, opposite the uracil: (ii) uracil in dimers is not a substrate for uracil N-glycosylase: and (iii) uracil-containing dimers are a substrate for DNA photolyase. An additional aim of this work was to determine the validity of these assumptions.

To examine these questions, we have constructed single-stranded vectors that contain a uniquely placed *cis-syn* or *trans-syn* U-U cyclobutane dimer. We find that the U-U dimers are, like their T-T counterparts (3,11), replicated with high accuracy when transfected into *E.coli*. The mutagenic properties of these photoproducts, however, differ from the T-T dimers in two ways: a greater ratio of C substitutions to A substitutions was induced by each isomer of the U-U dimer than by the corresponding T-T lesions, and the *trans-syn* U-U photoproduct, unlike the T-T isomer, failed to induce single base deletions. These observations point to the importance of photoproduct structure in determining mutagenic properties. In addition, we find that the U-U dimers are not substrates for uracil N-glycosylase, and that the *cis-syn* U-U dimer can be repaired by DNA photolyase.

MATERIALS AND METHODS

Strains

The *E.coli* strains used in this work were all derivatives of AB1157; SMH10 (F^+ *uvrA6* Δ*(pro-lac)*), SMH77 (F′ *lacZ* ΔM15 *pro*$^+$, Δ*(pro-lac) leu*$^+$) and SMH99, an *ung*$^-$ strain which was derived from SMH77 by Tn10 disruption of the *ung* (uracil N-glycosylase) gene. The SOS regulon was induced in SMH10 by exposure to 4 J m^{-2} germicidal UV, and in SMH77 and SMH99 by exposure to 40 J m^{-2}. The vector used was M13mp7L2, which is a derivative of M13mp7 in which the palindromic multiple cloning site has been lengthened (as described for the vector M13mp7L1; ref 5) and from which the amber mutations found in M13mp7 have been eliminated.

Construction of vectors with specifically placed cyclobutane dimer

Vectors with a specifically placed U-U or T-T dimer, or the photoproduct-free controls, were constructed as described previously (5, 15), with the exception that oligomers containing a U-U dimer were obtained by exposing 50 μg of the synthetic 11-mer 5′ GCAAGUUGGAG 3′, in 100 μl of anoxic water/acetone (30%, v/v), to sun lamp irradiation, filtered through polyester film (λ >315 nm) for 24 hr. The *cis-syn*, *trans-syn* and unreacted 11-mer were purified from the irradiated samples by reverse phase HPLC as described for the T-T dimers, and repurified twice before phosporylation and once after (15). The retention times of the U-U products were virtually identical to those of the T-T products, but the relative yields of the *cis-syn* and *trans-syn* isomers were about 3:1 with the U-U sequence, compared with about 9:1 for the T-T counterpart. The purity of the modified oligomers, as determined by a digestion assay (5), was > 99.5% and > 98% for the *cis-syn* and *trans-syn* isomers respectively.

Transfection

Cells were grown to a density of approximately $3-5\times10^8$ per ml in YT medium and were made competent by the $CaCl_2$ method, as described (15). Cells for SOS-induction were suspended in an equal volume of starvation buffer and exposed to 254 nm UV immediately prior to making them competent. For each transfection sample, 4 ng of construct (equivalent to 2−3 ng circular DNA) was added to 0.5 ml cells, giving a 10-fold excess of cells relative to construct molecules. These procedures were carried out in yellow light to inhibit photolyase activity.

Analysis of replication products

Well separated plaques on transfection plates were transferred to duplicate master plates seeded with a dilute lawn (2×10^6 cells of SMH10 or JM101), and grown overnight. One plate in each pair was blotted onto NEF-978X nylon membrane (DuPont), which was hybridized with a ^{32}P-labeled 15-mer complementary to the TT 11mer, and extending two base pairs on either side. The conditions for hybridization were as described (15) and under these conditions, the probe bound only to the non-mutant 11-mer sequence. Non-hybridizing vectors were sequenced by the dideoxy method, using a synthetic primer complementary to nucleotides 37 to 57 in the *lacZ* gene, counting from the base 3′ to the insertion site. A small number of the replicated construct molecules, generally less than 1% of the number in the control, contained either the complete hairpin sequence of the multiple cloning site, no 11-mer sequence or a G-G dinucleotide opposite the UU target site, indicating that they arose from replication primed by undenatured 51-mer scaffold. Phages of these types were excluded from the data.

Treatment of oligomers with uracil N-glycosylase and DNA photolyase

The susceptibility of 11-mer containing a U-U *cis-syn* dimer to uracil N-glycosylase was determined in reactions containing 2.5μg of this oligomer plus 2.5μg of unmodified U-U 11-mer in 70 mM Tris.HCl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol, and 13 U (1 U removes 1 pmole uracil/min) of *E.coli* enzyme (kindly provided by Dr Susan Wallace, University of Vermont), in a total volume of 50 μl. Reactions were incubated at 37 C for 1 h, followed by the addition of 3.5 μl of triethylamine and incubation for a further 10 min to cleave the abasic site, and the addition of 2 μl of glacial acetic acid to bring the solution approximately to neutral pH. Treated oligomer was analyzed by HPLC, using the method and buffers described by Banerjee et al. (15). The action of *E.coli* DNA photolyase (kindly provided by Gwendolyn and Aziz Sancar, University of North Carolina, Chapel Hill) on the same mix of oligomers was carried out as described previously (15), and the DNA analyzed by the same HPLC method.

RESULTS

Single-stranded, M13mp7-based constructs, carrying either a *cis-syn* or a *trans-syn* U-U cyclobutane dimer located at a unique site, were constructed by the method of Banerjee et al. (15). In

this procedure, synthetic 11-mers containing the photoproduct are first produced and purified, and the modified oligomers are then ligated directly into the single-stranded vector. U-U Dimers were induced at a unique site in the oligomer by a photosensitized reaction that employed acetone (30% v/v) rather than acetophenone, which was used to induce T-T dimers. Different aliquots from samples of competent cells of *E.coli* strains SMH10 (*uvrA6*), SMH77 (*uvr*$^+$ *ung*$^+$) and SMH99 (*uvr*$^+$ *ung*$^-$), were transfected with equal amounts of construct that contained one or other of the U-U photoproducts, one or other of their T-T counterparts, or a lesion-free T-T sequence, to act as control. Both SOS-induced and uninduced cells were used. Plaques resulting from the transfection were counted and random samples of them taken from SOS-induced cells of strains SMH10 and SMH99 for analysis (15) of the nucleotide sequence in a region of ~ 150 nucleotides, spanning the photoproduct target site. Sequence analysis was not carried out on plaques from uninduced cells because very few were obtained in the absence of SOS induction. These data were used to estimate photoproduct bypass frequency (that is, the proportion of molecules in which replication has proceded past the site of the photoproduct in the single-stranded construct), the error frequency of bypass, and the mutation spectrum, which are the parameters defining lesion mutagenicity. Bypass frequency was estimated from the number of plaques obtained with photoproduct-containing construct, normalized to the number of plaques in the control, using uninduced cells. This value accurately represents bypass frequency because modified and control oligomers are ligated into the vector with equal efficiency (15), the uptake of modified and control construct into competent cells is likely to be equal (16), and no known repair process can act on these single-stranded vectors prior to replication. The error frequency of bypass and mutation spectrum were obtained directly from the sequence analysis. Material for analysis was taken randomly, without the use of reporter genes or sequences, ensuring that the normal and all mutant sequences could be detected efficiently and without bias. Such data are subject only to the constraint that the construct must be replicated.

Different spectra and frequencies of mutations are induced by U-U and T-T dimers

Although the overall frequencies and types of mutations induced by the U-U and T-T dimers were fairly similar (Table 1), as might be expected from their similarity in structure, there were nevertheless significant differences between the mutagenic properties of both of the pairs of the corresponding isomers. With each of the four photoproducts, A-A was preferentially incorporated opposite the lesion, yielding phage containing a T-T sequence in about 95% and 85% of the progeny resulting from bypass of the *cis-syn* and *trans-syn* isomers, respectively. With each of the photoproducts, substitution mutations arose predominantly from the incorporation of either T or G, principally opposite the 3′ nucleotide of the dimer with the *cis-syn* isomers, and opposite the 5′ nucleotide for the *trans-syn* photoproducts, yielding T-A and T-C, and A-T and C-T sequences respectively. The uracil isomers differ from their thymine counterparts, however, with respect to the ratios of the T and G misincorporation events. For the *cis-syn* isomers these ratios were > 4:1 for the T-T dimer, but only about 2:1 for the U-U lesion, a difference that is significant ($p = 0.03$). With the *trans-syn* isomers the change in the ratios was not only in the same direction, but was also even greater, from 4:1 with the T-T photoproduct to 1:2 with the U-U lesion, a difference that is highly significant ($p = 0.001$). The consistency of the direction in the changes of these ratios strongly suggests that the presence or absence of the methyl group at C-5 is itself responsible for the altered pattern of substitution, rather than a less specific difference in stereochemical structure of the U-U and T-T photoproducts.

In addition to this alteration in substitution mutagenesis, the uracil and thymine photoproducts also differ with regard to the induction of targeted single base deletions by the *trans-syn* isomers; with the T-T lesion, such frameshifts were the most abundant type of mutation (33/604 sequences analyzed), but none at all were found with the U-U dimer (0/560 sequences analyzed), a highly significant difference ($p << 0.001$). This result does

Table 1. Sequence at U-U or T-T[a] photoproduct target site in M13mp7L2 vectors replicated in SOS-induced cells of *E.coli* strain SMH10 (*uvrA6 ung*$^+$)

Sequence at target site	Number observed (percent)			
	cis-syn dimers		*trans-syn* dimers	
	U-U	T-T	U-U	T-T
T-T	891 (94.7)	2443 (93.7)	476 (85.0)	536 (88.7)
A-T	0	0	22 (3.9)	16 (2.6)
C-T	0	6 (0.2)	49 (8.8)	4 (0.7)
G-T	0	0	2 (0.4)	0
T-A	31 (3.3)	130 (5.0)	5 (0.9)	0
T-C	16 (1.7)	28 (1.1)	3 (0.5)	1 (0.2)
T-G	0	0	1 (0.2)	0
ΔT	0	0	0	33 (5.5)
other[b]	3 (0.1)	1 (0.04)	2 (0.4)	14 (2.3)
Total	941	2608	560	604
error freq. (%)	5.3	6.3	15.0	11.3

[a]Data from Lawrence et al. (3).
[b] For U-U *cis-syn*; one T-T→C-A, two Δ T-T: for U-U *trans-syn*; one Δ C in the 11-mer and one Δ 300 nucleotides, including the multiple cloning site.

Table 2. Sequence at U-U target site in M13mp7L2 vectors replicated in SOS-induced cells of strains SMH77 (*uvr*$^+$ *ung*$^+$) and SMH99 (*uvr*$^+$ *ung*$^-$)

Sequence at target site	Number observed (percent)			
	SMH77 (*ung*$^+$)		SMH99 (*ung*$^-$)	
	U-U control	U-U control	U-U *cis-syn*	U-U *trans-syn*
T-T	89 (61.4)	598 (100)	136 (92.5)	125 (83.9)
A-T	9 (6.2)	0	0	7 (4.7)
C-T	24[a] (16.6)	0	0	14 (9.4)
G-T	1 (0.7)	0	0	2 (1.3)
T-A	1 (0.7)	0	7 (4.8)	0
T-C	13 (9.0)	0	4 (2.7)	1 (0.7)
T-G	0	0	0	0
A-A	1 (0.7)	0	0	0
A-C	1[a] (0.7)	0	0	0
C-A	3 (2.1)	0	0	0
other[b]	3 (2.1)	0	0	0
total	145	598	147	149

[a] One sequence in each class also had a deletion of the fourth nucleotide (ΔA4) in the 11-mer sequence, 5′ GCAA-GUUGGAG 3′.
[b] Two ΔA4 mutations and one ΔT frameshift.

not appear to be the consequence of a contaminating, non-dimer, photoproduct that induces the frameshifts, and which is present in the samples of T-T but not U-U 11-mer. Evidence that the T-T *trans-syn* dimer itself induced these mutations was obtained by exposing samples of 11-mer containing this photoproduct to 0,4 or 10 kJ/m^2 of 254 nm UV, followed by ligation of the oligomers into the vector, transfection of construct into SOS-induced and uninduced cells of SMH10, and nucleotide sequence determination of progeny phage. The principal behind this test is the unique property of cyclobutane dimers to be both formed and split by UV irradiation, resulting in an equilibrium yield. Exposure of the dimer-containing 11-mer to 254 nm UV reduces the proportion of molecules with dimer from 100% to about 15–20%, the remaining 80–85% of the molecules being the normal, photoproduct-free species (15). If the single T deletions were induced by a contaminating non-dimer photoproduct, the frequency of these frameshifts would be unaffected by the UV-induced dimer splitting, whereas the frequency of substitutions would decrease. Conversely, if the frameshifts are induced by the dimer, the frequency of both frameshifts and substitutions should coordinately decrease. It was this latter result that was observed; after irradiating dimer-containing 11-mer with 4 kJ/m^2, one 5 substitution was found and 3 targeted deletions, among a total of 870 phage analyzed. Dimer-containing 11-mer that was not UV irradiated gave 18 mutants with 5 substitutions and 16 mutants with targeted single T deletions among 333 phage analyzed. Evidence that the UV treatment used effectively split the dimers as expected was provided by the much enhanced transformation frequencies found after dimer splitting. Construct made with UV-irradiated T-T *trans-syn* 11-mer produced about 85% as many plaques as the control construct in uninduced cells, and as many plaques as the control in SOS-induced cells, the same result being found when fluences of either 4 or 10 kJ/m^2 were used. When the dimer-containing 11-mer was not irradiated, the corresponding values were 9% and 19%. Although the UV treatment effectively split the dimers, it did not itself appear capable of inducing many photoproducts; irradiation of photoproduct-free 11-mer with 10 kJ/m^2 produced 1 mutation, a 3′T→TA substitution perhaps induced by a *cis-syn* dimer, among 300 phage analyzed.

The sequence spectrum for the U-U photoproducts was independent of the *ung* phenotype of the host cells suggesting that uracil in dimers is not a substrate for the uracil N-glycosylase; plaques obtained from the *ung*$^-$ strain SMH99, which lacks this activity, gave similar types of frequencies of substitutions (Table 2). Apart from this evidence, it is also clear that the abasic sites that would result from such base excision have substantially different mutagenic properties (Table 2). Phage resulting from transfection of SMH77 (*ung*$^+$) with construct carrying the normal, lesion-free U-U sequence exhibit a high mutation frequency (56/145, 38.6%), with substitutions at the 5′ site (23.4%), the 3′ site (9.7%), and tandem double substitutions at both sites (3.4%). The ratios of nucleotide incorporation at each site are significantly different to those seen with either of the U-U dimers. In view of previous sequence data for single abasic lesions at one or the other of these two positions (17), these results indicate that endogenous uracil N-glycosylase acting on the U-U sequence removes at least one of these bases with high efficiency before replication. Among phage replicated in SMH99 (*ung*$^-$), all of the 598 sequences examined were of the T-T type, indicating that in the absence of base excision both uracil residues were accurately replicated.

U-U dimer bypass efficencies

Sets of matched samples of construct were transfected into uninduced or SOS-induced cells of strains SMH10 (*uvrA6 ung*$^+$), SMH77 (*uvr*$^+$ *ung*$^+$) or SMH99 (*uvr*$^+$ *ung*$^-$), at a ratio of approximately 0.2 molecules of construct per cell. In the first series of experiments (A in Table 3), each set of constructs used included five matched samples carrying one or other of a *cis-syn* or *trans-syn* dimer at a U-U or T-T target site, or a photoproduct-free T-T control sequence. However, in one additional initial experiment with SMH10, constructs carrying the T-T photoproducts were omitted. In the second series (B in Table 3), matched sets of constructs carrying T-T and U-U control (lesion-free) sequences were used. As shown in Table

Table 3. Bypass frequency, estimated from the number of plaques normalized to the number in uninduced cells transfected with T-T control construct, resulting from (A) transfection with vectors carrying a single U-U or T-T dimer or (B) the U-U control sequence

Photoproduct	Percent bypass					
	SMH10 (*uvr*$^-$ *ung*$^+$)		SMH77 (*uvr*$^+$ *ung*$^+$)		SMH99 (*uvr*$^+$ *ung*$^-$)	
	− SOS	+ SOS	− SOS	+ SOS	− SOS	+ SOS
(A) T-T control	100.0	105.2	100.0	99.6	100.0	124.4
U-U *cis syn*	0.8	18.9	0.1	36.3	0.0	35.9
T-T *cis syn*	0.7	26.5	0.3	34.6	0.2	45.2
U-U *trans syn*	0.2	8.8	0.1	34.1	0.0	29.1
T-T *trans syn*	7.7	14.0	4.6	31.5	4.0	28.2
(B) T-T control	–	–	100.0	109.4	100.0	124.0
U-U control	–	–	0.1	11.2	95.4	113.5

Data are for transfections with construct and, except with SMH99 in (B), exclude plaques resulting from transfection with uncut vector, scaffold priming, or religation without 11−mer insert. Such events were detected by sequence analysis, and collectively were ~ 1% of the control plaque number. The table entries are the averages of data from 3 independent experiments using matched sets of constructs, except for SMH10 where results from a fourth experiment using only the uracil photoproducts and T-T control are also included. SOS-induction of SMH10 cells was achieved by exposing them to 4 J m^{-2} of 254 nm UV, while SMH 77 and SMH99 cells were exposed to 40 J m^{-2} UV irradiation.

3, the U-U and T-T photoproducts inhibit replication to a similar extent, and in most respects exhibit similar bypass characteristics. With each photoproduct, bypass frequencies were very low in uninduced cells, and were much increased by SOS-induction. Bypass frequencies in excision-proficient strains SMH77 and SMH99 were much higher than in excision deficient SMH10, perhaps reflecting more efficient SOS-induction.

The presence of uracil N-glycosylase appeared to have little influence on bypass, however, consistent with the hypothesis that uracil in dimers is protected from the enzyme. If uracil is not protected in this way (Table 3, B), abasic sites are efficiently and promptly produced in > 99% of viral genomes before replication, as shown by the almost complete block to replication in uninduced cells, and these are bypassed with low efficiency (11%) in SOS-induced cells. This efficiency is nevertheless higher than the 5%−7% seen previously for a single abasic site in SMH10 (17), a particularly significant increase because a proportion of the present molecules are likely to carry a tandem double abasic site. This higher bypass frequency is again likely to result from more efficient SOS-induction.

The only consistent difference in bypass properties between the U-U and T-T photoproducts concerns the *trans-syn* isomers. As found previously (15), the T-T *trans-syn* dimer was bypassed to a small but significant extent in the absence of SOS-induction, whereas the corresponding U-U photoproduct showed almost no bypass in uniduced cells. The difference does not appear to reflect contamination of the photoproduct-containing species with unmodified construct which in the case of the U-U, but not T-T sequence, would be converted to a non-bypassable abasic site. Further, digestion analysis (15) suggests that the oligomer with the T-T photoproduct is more pure (>99%) than the equivalent U-U 11-mer (>98%). More probably therefore, this, like the capacity to induce single nucleotide deletions, is an inherent property of the T-T but not U-U lesion.

Action of uracil N-glycosylase and DNA photolyase on uracil *cis-syn* dimers *in vitro*

To determine if the U-U *cis-syn* cyclobutane dimer is a substrate for DNA photolyase or uracil N-glycosylase, an equimolar mixture of 11-mers with and without the photoproduct was treated with these enzymes *in vitro*, followed by injection of the whole sample and analysis by reverse-phase HPLC. As shown in Figure 1C, photolyase converts the dimer-containing species to an unmodified oligomer (Figure 1A) in a blacklight-dependent reaction (Figure 1B). Treatment of an identical mixture of 11-mers with uracil N-glycosylase followed by a treatment with mild alkali resulted in cleavage of the unmodified 11-mer into two smaller, unresolved, fragments that elute with the void volume (Figure 1D). The intact dimer-containing species was quantitatively recovered, however, showing that it is not a substrate for this enzyme.

DISCUSSION

We have determined the bypass frequencies, error frequencies of bypass, and mutation spectra in *E.coli* cells for the *cis-syn* and *trans-syn* isomers of a U-U cyclobutane dimer, and compared these with similar data previously established for their T-T counterparts (3), in order to examine the influence of the chemical structure of the template on the mutagenic properties of these photoproducts. We have also investigated these properties, together with the susceptibility of the U-U *cis-syn* dimer to DNA photolyase and uracil N-glycosylase, because such photoproducts may arise by deamination of cytosine within dimers (18), and therefore may contribute to the spectrum of UV-induced mutations.

We find that whereas the mutagenic properties of the U-U and T-T photoproducts are in many ways identical, they nevertheless differ significantly in three respects, pointing to an influence of the thymine C-5 methyl group, the only structural feature by which they differ. The first difference in properties concerns the proportions of substitutions induced at the major site for such mutations, which is the 3′ nucleotide for the *cis-syn* dimers and the 5′ site for the *trans-syn* isomers. Substitutions of A and C but, for the most part, not G occur at these sites with each photoproduct, but both U-U isomers induce a higher proportion of C substitutions than their T-T counterparts; the ratios of A to C substitutions were 2:1 versus 4.5:1 for U-U and T-T *cis-syn* dimers, and 1:2 versus 4:1 for the corresponding *trans-syn* lesions. The second difference in mutagenic properties between uracil and thymine dimers is found in the induction of targeted

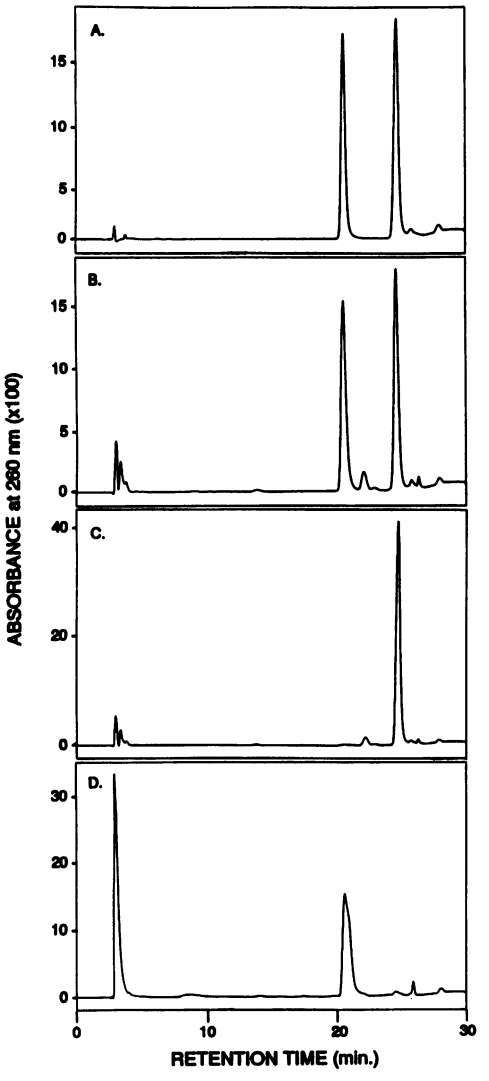

Figure 1. HPLC chromatograms of a mixture of 11-mer 5′ GCAAGUUGGAG 3′ carrying a U-U *cis-syn* cyclobutane dimer and the corresponding photoproduct-free U-U 11-mer, treated with DNA photolyase or uracil N-glycosylase, or untreated. **A**, untreated control. The peak at 20.5 min is the dimer-containing species, and that at 24.5 min is the photoproduct-free oligomer. **B**, oligomer treated with photolyase in photolyase buffer, and kept in the dark to prevent photoreactivation. **C**, as in B, but exposed to photoreactivating blacklight for 60′, which converts the dimer-containing 11-mer to the dimer-free species and increases the size of this peak. **D**, oligomer treated with uracil N-glycosylase in glycosylase buffer, followed by alkali to cleave the oligomer at the abasic sites. The dimer-free 11-mer is split into short oligomers that elute at 5 min, but the dimer-containing species quantitatively remains unsplit.

single nucleotide deletions by the T-T but not U-U *trans-syn* photoproduct. The role of the T-T lesion, rather than some contaminating photoproduct, in producing the frameshifts was demonstrated by the concerted decrease in the frequency of both substitutions and frameshifts concomitant with dimer splitting by UV irradiation. The T-T dimer also induced near-targeted +1 frameshifts (9/14 designated as other in Table 1, see ref. 15) not found with the U-U lesion. The missing frameshift mutations appear to have been replaced by an increase in substitutions, chiefly U→C, induced by the U-U dimer (Table 1). The third difference in mutagenic properties concerns the occurrence of a small but significant frequency of bypass of the T-T *trans-syn* dimer, though not of its U-U counterpart, in uninduced *E.coli* cells. Since this SOS-independent bypass was found previously (15) to induce a low frequency of mutations that were exclusively single nucleotide deletions, the same mechanism may be responsible for both phenomena.

The occurrence of these differences in mutagenic properties points to the importance of a feature of template structure, the presence of an exocyclic methyl group at C-5 in thymine and its absence in uracil, in determining such properties. By the same token, these data do not support a view that nucleotide insertion depends solely on a polymerase-based A-rule (2), with template structure being of minimal importance. The way in which the template influences mutation induction is not known, though different mechanisms are likely to cause substitutions and frameshifts. Substitutions may arise from the formation of hydrogen bonded base-pairs involving T·T or U·T and T·G or U·G, because formation of a cyclobutane ring does not inherently prevent base-pairing; NMR analysis of oligomer duplexes containing a *cis-syn* (19,20) and *trans-syn* (20) T-T dimer in one strand shows each thymine base-paired with adenine except for the 5′ nucleotide in the *trans-syn* isomer, which is permanently unstacked and in *syn* with respect to the glycosyl bond. Such base-pairing is likely to be responsible for the high accuracy of replication past these photoproducts. Even though the A·U association may be a little weaker than that for A·T (21), this does not reduce the accuracy. Frameshift mutations, and also a small amount of SOS-independent bypass, may arise because the C-5 methyl group, within the distorted structure of the dimer, slightly reduces the inhibition of chain elongation, though at the cost of interfering with the ability of the DNA polymerase to recognize template positions appropriate for base insertion.

A second reason for studying uracil dimers is that, as a result of the deamination of cytosine in these photoproducts, they may contribute to the spectrum of UV-induced mutations. Saturation of the 5,6 double bond in these lesions much enhances the rate of deamination, though exactly what this rate is *in vivo* is still a matter for debate (8−10). It is clear, however, that if such deamination takes place it is likely to much enhance the mutagenicity of the dimers. As shown in Table 1, cyclobutane dimers are inherently replicated with high accuracy and because this is a property of their structure, it is also likely to be true, in the absence of deamination, for the cytosine-containing variety. Because guanine is almost never inserted opposite uracil in the photoproduct (Table 1), deamination will be almost 100% mutagenic during dimer bypass. Results supporting this conclusion have also been obtained recently by others in a different sequence context (22). The mutagenic potential of such deamination is also high because the presence of the photoproduct inhibits the action of the endogenous uracil N-glycosylase, which would otherwise rapidly remove the deamination product; under the experimental conditions used, uracil not protected in this way was efficiently excised before phage replication took place (Tables 2 and 3). Lesion-inhibition of uracil excision is analogous to the prevention of the correction of spontaneously occurring mismatched base-pairs by photoproducts, which was suggested previously (3). Protection from excision would be lost after photoreactivation, however, because the U-U *cis-syn* dimer is a substrate for DNA photolyase (Figure 1). Photoreactivation is therefore likely to much reduce deamination-dependent mutagenesis, except perhaps at sites where the dimer is so close

to a blocked replication fork that the glycosylase has insufficient time to act before replication takes place (6).

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