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Evidence for lack of DNA photoreactivating enzyme in humans

(thymine dimer/skin cancer/mammals/reptiles)

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ABSTRACT **Photoreactivating enzyme (DNA photolyase; deoxyribocyclobutadipyrimidine pyrimidine-lyase, EC 4.1.99.3) repairs UV damage to DNA by utilizing the energy of near-UV/visible light to split pyrimidine dimers into monomers. The enzyme is widespread in nature but is absent in certain species in a seemingly unpredictable manner. Its presence in humans has been a source of considerable controversy. To help resolve the issue we used a very specific and sensitive assay to compare photoreactivation activity in human, rattlesnake, yeast, and *Escherichia coli* cells. Photolyase was easily detectable in *E. coli*, yeast, and rattlesnake cell-free extracts but none was detected in cell-free extracts from HeLa cells or human white blood cells with an assay capable of detecting 10 molecules per cell. We conclude that humans most likely do not have DNA photolyase.**

Pyrimidine dimers (Y◇Y) are one of the major DNA photoproducts induced by solar irradiation of biological systems. These lesions can be eliminated from DNA by photoreactivation or excision repair (1). Excision repair has been found in all species tested. In contrast, photoreactivation (splitting of Y◇Y by an enzyme called DNA photolyase) is not universal, and its distribution among various species, superficially at least, does not make teleological sense. Thus, the enteric bacterium *Escherichia coli* is photoreactivable but the soil bacterium *Bacillus subtilis* is not; the budding yeast *Saccharomyces cerevisiae* is photoreactivable but the fission yeast *Schizosaccharomyces pombe* is not (2). Similarly, primary fibroblasts from the rattlesnake (*Crotalus horridus*) remove thymine dimers (T◇T) by photoreactivation efficiently, whereas those from the garter snake (*Thamnophis* sp.) do not (3).

When mammalian cells were tested, initially it was claimed that marsupials were capable of photoreversing Y◇Y but that placental animals, including humans, were not (4, 5). However, subsequent research on this subject yielded conflicting results. In a series of papers, it was reported that human leukocytes as well as human and murine cells in culture contained photolyase (6–8), that fibroblasts from a patient of xeroderma pigmentosum (XP) complementation group B were completely defective in the enzyme (7), and that cell lines from other XP complementation groups, including the XP variant, had diminished photoreactivation activity (7, 8). Other studies failed to detect photoreactivating enzyme (DNA photolyase) in human or murine cell lines (9). However, evidence was presented suggesting that expression of the enzyme is tissue-specific and that in tissue culture the expression is influenced by the growth medium (10, 11), which may account for the failure of some researchers to detect photoreactivation in humans and other placental mammals.

The presence or absence of photolyase in humans is important from the standpoint of public health (12). Therefore, we have reexamined this question, employing new methodology that has become available within the last few years—specifically, the ability to make large quantities of substrate with a single T◇T at a defined position (13). Such a substrate makes it possible to detect very low levels of photolyase. Further, since the photoproduct is chemically incorporated into DNA the ambiguity arising from the nature of the photoproduct affected by photoreactivation treatment is eliminated. Our results with this substrate system and extracts from cultured human cells and from white blood cells (WBCs) taken from volunteers reveal that humans do not have a photoreactivating enzyme.

MATERIALS AND METHODS

Materials. *Mse* I restriction endonuclease and polynucleotide kinase were from New England Biolabs. Poly(dA) and $(dT)_{12-18}$ were from Pharmacia. Sodium pyruvate and β-NADH were from Sigma. [γ-^{32}P]ATP (7000 Ci/mmol; 1 Ci = 37 GBq) was from ICN. Purified *E. coli* DNA photolyase was prepared as described (14).

Photolyase Substrates. Two types of substrates were used: a synthetic oligomer with a single T◇T, and UV-irradiated pBR322. The synthetic substrate was prepared by the "building block" method (13) and was a gift of J.-S. Taylor and D. L. Svoboda (Washington University, St. Louis). The oligomer containing T◇T was 20 nucleotides in length: 5′-GCTCGAGCTAT◇TAACGTCAG-3′. To prepare substrate, this 20-mer was 5′-end-labeled, annealed to the complementary strand, and digested with *Mse* I (which incises at T↓TAA) to eliminate dimer-free contaminant. The resulting DNA was >99% pure with regard to T◇T content. The concentration of the substrate used in Figs. 2 and 3 was determined by Cerenkov counting and by taking into account that 50% of the label used in the kinase reaction was incorporated into DNA. The specific activity of this substrate was 6835 Ci/mmol.

The pBR322 substrate was prepared by irradiating the plasmid with 254-nm light and the number of Y◇Y per plasmid molecule was determined by a transformation assay (14).

Cell-Free Extracts (CFEs). Extracts were prepared from *E. coli* (15) and *S. cerevisiae* (16) by established procedures. Extract from rattlesnake (*C. horridus*) was prepared as follows. Diamond-head rattlesnake muscle was obtained from Aries (Dallas). The small pieces of frozen muscle (typically 1–2 g) were homogenized in 10 vol of 50 mM Tris·HCl, pH 7.5/100 mM NaCl/10 mM EDTA/10 mM 2-mercaptoethanol/0.25 M sucrose/10 mM leupeptin/10 mM aprotinin/10 mM phenylmethanesulfonyl fluoride. The debris was removed by centrifugation at 32,000 × *g* for 20 min and then at 120,000 × *g* for 2 hr. Proteins were precipitated with 55% saturated ammonium sulfate, suspended in 50 mM

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Abbreviations: CFE, cell-free extract; WBC, white blood cell; Y◇Y, pyrimidine dimer(s); T◇T, thymine dimer(s); LDH, lactate dehydrogenase; Pol ε, DNA polymerase ε; XP, xeroderma pigmentosum.

Tris·HCl, pH 7.4/10 mM EDTA/10 mM dithiothreitol/20% (vol/vol) glycerol, and dialyzed against the same buffer with 50% glycerol. Aliquots of 200 μl were frozen in dry ice/ethanol and stored at −70°C until use.

Human CFE was prepared from the HeLa cell line and from WBCs. The preparation of whole CFE from HeLa cells has been described (17–19). For small-scale preparations of CFE from WBC, 35 ml of blood was withdrawn from each of four workers in our laboratory. The WBCs were separated on Ficoll-Paque R (Pharmacia LKB) gradients according to the manufacturer's procedure. The milky buffy coat between the plasma layer and the red blood cell layer was taken and CFE was prepared as described (17–19). The yield of protein from the WBCs of the four individuals ranged from 9.6 to 71.1 μg. For large-scale preparation of human WBC extracts, 2 liters of freshly drawn blood was obtained from the North Carolina Red Cross Center and extract was prepared by the same procedure. The yield was 27 mg at a concentration of 7.4 mg/ml. All CFEs were dialyzed against TDEG buffer (50 mM Tris·HCl, pH 8.0/1 mM dithiothreitol/1 mM EDTA/17% glycerol), aliquoted, frozen in dry ice/ethanol, and stored at −80°C until use.

Often enzymes show suboptimal activity in CFE because of interference by other proteins in the crude extract. Therefore, we also partially purified the human CFE before testing for activity. The concentrated CFE (10 mg at 14 mg/ml) was loaded onto a 3-ml DEAE-agarose column equilibrated with 0.1 M NaCl in TDEG buffer. The column was washed with the same buffer, which eluted about 80% of the proteins (fraction D1), and the bound proteins were eluted with 800 mM NaCl in the same buffer (fraction D2). D2 was dialyzed into 0.1 M NaCl in TDEG and both D1 (7.2 mg/ml) and D2 (3.4 mg/ml) were stored in aliquots at −80°C until use.

Photolyase Assays. Two assays were employed, a chemical/coupled enzyme assay and a biological/transformation assay. In the chemical assay, the reaction mixture (50 μl) contained 50 mM Tris·HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA, 20 mM dithiothreitol, 7% glycerol, 10 μg of bovine serum albumin, 1.0 nM 20-mer (T◇T), and the indicated amounts of CFE. The reaction mixture (at 23°C) was exposed to photoreactivating light from two Vivitar 5000 camera flash units flashed 0–600 times at about 4-sec intervals simultaneously with illumination with a General Electric black light (λ_{max} 365 nm) at 2 mW/cm^2 as indicated. Following photoreactivation, 1 μl of proteinase K (10 mg/ml) and 2.5 μl of 10% SDS were added and the samples were incubated for 30 min at room temperature. The DNA was then extracted with phenol/chloroform, ethanol-precipitated, and suspended in 20 μl of 10 mM Tris·HCl, pH 7.5/50 mM NaCl/10 mM $MgCl_2$/1 mM dithiothreitol. Two units of *Mse* I was added and the mixture was incubated at 37°C for 1 hr. The digested DNA was analyzed by electrophoresis in 12% polyacrylamide sequencing gels. The gels were scanned with an Ambis scanner (Ambis Systems, San Diego) to quantify the level of repair, which is indicated by the fraction of 20-mer which is converted to 11-mer by restriction enzyme digestion.

Photoreactivation was also measured with natural DNA by the transformation assay (14). The substrate was pBR322 (50 μg/ml) irradiated with 250 J/m^2 of 254 nm from a General Electric germicidal lamp. This irradiation produced 5.5 lethal lesions per molecule. The photoreactivation assay was conducted as follows. The reaction mixture (50 μl) contained 50 mM Tris·HCl (pH 7.4), 50 mM NaCl, 10 mM dithiothreitol, 0.15 pmol of pBR322 (0.83 pmol of UV photoproducts), and 20 μg of CFE from *E. coli*, rattlesnake, or human WBCs. The samples were photoreactivated at a fluence rate of 0.5 mW/cm^2 for 0–70 min with a General Electric black light (λ_{max} 365 nm) filtered through an Oriel 290-nm-cutoff filter. Following photoreactivation, the DNA was deproteinized and suspended in 25 μl of water. The level of Y◇Y repair was tested by transformation (14).

Other Assays. The quality of animal CFEs was tested by assaying for DNA polymerase ε (Pol ε) (20) and lactate dehydrogenase (LDH) (21).

RESULTS

Test for Photolyase in CFEs from Various Species. Since some enzymatic activities, particularly when present in low levels, are obscured by other proteins present in CFEs, we first wished to establish that photolyase can be detected in CFEs from organisms known to have the enzyme at different levels. *E. coli* contains 10–20 molecules of photolyase per cell, and *S. cerevisiae* contains 100–200 molecules per cell (2). As an animal control we used rattlesnake, which is known to be efficient in photoreactivation (3). For humans, we used leukocytes, since it has been reported that nonlymphocytic WBCs are a good source of the enzyme (6, 7). However, it has also been reported that as much as a 30-fold difference in photolyase level can be found between individuals (22). Therefore, we tested WBCs from four volunteers who were chosen to represent different ethnic groups, as well as WBCs from a blood bank.

CFEs from *E. coli*, yeast, and rattlesnake photorepaired T◇T efficiently (Fig. 1). The level of photoreactivation, when compared with that achieved with pure *E. coli* photolyase, and assuming similar catalytic efficiencies for the other enzymes, is consistent with photolyase constituting about 10^{-6} to 10^{-5} of total cellular proteins in all three organisms. In contrast to these species, CFEs from WBCs of four individuals of Oriental (DH and YL), Croat/Irish (JR), and Anglo-Saxon (TM) backgrounds failed to yield any detectable

Source	–	PL	EC	SC	CH	DH	YL	TM	JR	EC	EC+JR
Protein (μg)	0	2.7×10^{-4}	42	44	6	19	4.8	4.5	13	4.2	4.2+13
% T<>T Repaired	0	27	13	23	16	0	0	0	0	3.6	2.3
Lane	1	2	3	4	5	6	7	8	9	10	11

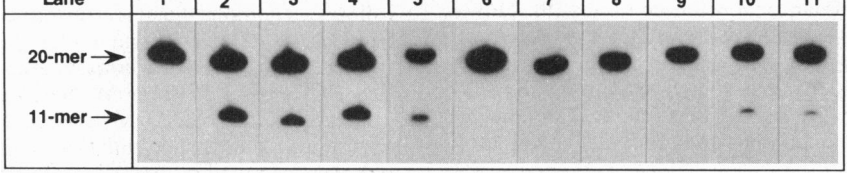

FIG. 1. Repair of T◇T by CFEs from various species. CFEs were mixed with 20-mer (T◇T) in 50 μl of reaction buffer and exposed to 200 camera flashes in ≈60 min while being exposed to a black light (λ_{max} 365 nm) at 2 mW/cm^2. After deproteinization the DNA was digested with *Mse* I and separated in a 12% polyacrylamide gel. As a control the same assay was conducted with 0.27 ng of purified *E. coli* photolyase (PL). CFE sources: EC, *E. coli*; SC, *S. cerevisiae*; CH, *C. horridus*; DH, YL, TM, and JR are the initials of the human donors of WBCs. Positions of *Mse* I-resistant full-length substrate (20-mer) and the *Mse* I-generated 11-mer product from repaired DNA are indicated. The sample in lane 1 was exposed to 200 flashes and digested with *Mse* I, which yielded 0.2% 11-mer. This was taken as the background value in our calculations.

photorepair. The lack of repair with human CFEs was not due to the presence of a specific photolyase inhibitor in the human extracts. Mixing of CFEs from human and *E. coli* resulted in only partial inhibition of the *E. coli* enzyme (Fig. 1, lane 11), as has been seen before with oligomer substrates (19, 23, 24). In this assay we could easily have detected as little as 1% repaired substrate, or about 4% of the repair we observed with 2.7×10^{-4} μg of *E. coli* photolyase. Since human CFEs ranging from 4.5 to 19 μg failed to give a repair signal even of this magnitude, if human WBCs contain photolyase it must constitute less than $(2.7 \times 10^{-4} \times 4 \times 10^{-2})/19 \approx 5 \times 10^{-7}$ of total cellular proteins.

Effect of Protein Concentration. An *in vitro* system for human nucleotide excision repair has been developed (17, 18). In this system it was found that for optimal activity the CFE protein concentration was critical and needed to be between 1 and 2 mg/ml (17). Considering the possibility that there might be a similar concentration dependence for photorepair, we conducted photoreactivation experiments with various concentrations of CFEs from *E. coli*, rattlesnake muscle, and human WBCs (Fig. 2). The level of photoreactivation increases linearly with the amount of rattlesnake extract until the protein level becomes saturating, as is expected for a bimolecular reaction. With *E. coli* CFE at high protein concentrations some nonlinearity is observed due to degradation of DNA by nonspecific nucleases. Nevertheless, at all concentrations tested photorepair is observed unambiguously. In contrast, no activity is detected with human CFE in the range of 22–560 μg of protein. These data are consistent with the conclusion that human WBCs do not contain photolyase at a level exceeding 10^{-7} of total cellular proteins.

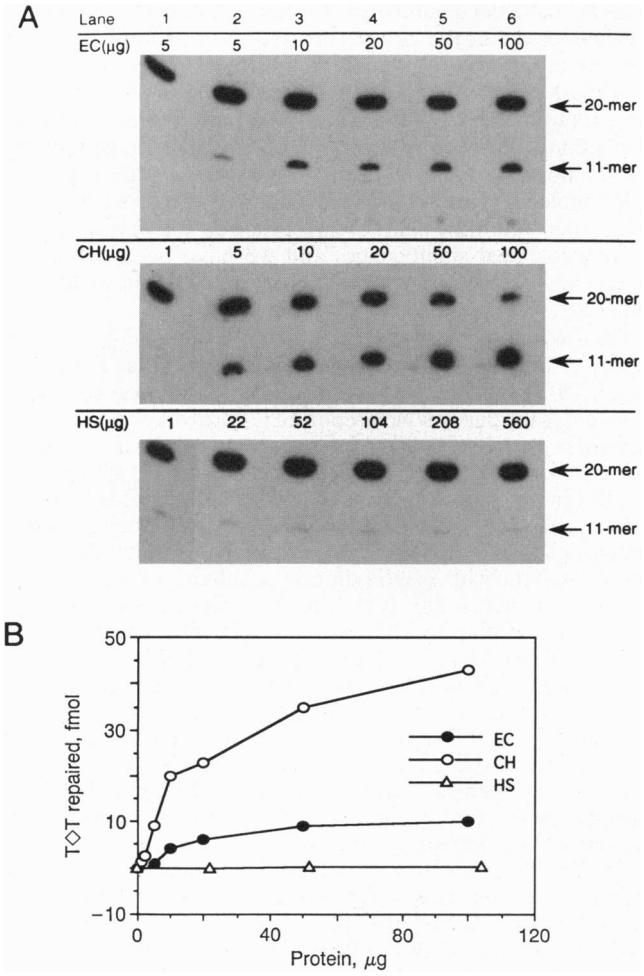

FIG. 2. Repair of T◇T by *E. coli* (EC), rattlesnake (*C. horridus*, CH), and human (*Homo sapiens*, HS) CFEs as a function of protein concentration. The 50-μl reaction mixtures contained 50 fmol of substrate and the indicated amounts of CFE protein and were exposed to 200 flashes over a 60-min period as in Fig. 1. (*A*) Autoradiograms of 12% polyacrylamide gels. Lanes 1, control reactions in which samples containing the indicated amounts of protein were kept in the dark and then treated with *Mse* I to determine the basal level of 11-mer, which was 0.8% for EC, 1% for CH, and 0.8% for HS. These values have been subtracted from the values in lanes 2–6 to calculate the level of repair. (*B*) Average of data from two experiments, including the one shown in *A*.

Photoreactivation Kinetics. To further define the upper limit of photolyase activity that might be present in human WBCs, we conducted kinetic experiments. Fig. 3 shows that 49 μg of *E. coli* extract and 6 μg of rattlesnake extract yielded similar kinetics. With human WBC extract, at 25 μg (data not shown) and 50 μg per reaction no activity could be detected with up to 600 camera flashes. Considering that *E. coli*, which contains photolyase at a level of 3×10^{-6} of total cellular proteins (see Fig. 1), gave an easily detectable signal with 100 flashes while human CFE failed to yield a signal (5% of the repair seen with *E. coli* would have been detectable) even with 600 flashes, we conclude that the level of photolyase in human CFE from WBC must be less than $(3 \times 10^{-6} \times 5 \times 10^{-2} \times 10^2$ flashes$)/6 \times 10^2$ flashes $= 2.5 \times 10^{-8}$ of total cellular proteins.

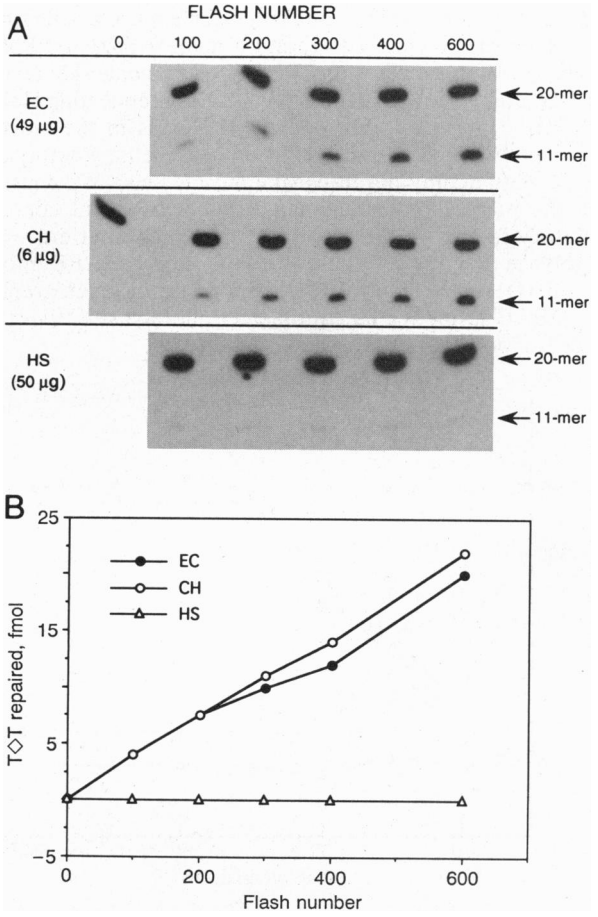

FIG. 3. Repair of T◇T by *E. coli* (EC), rattlesnake (CH), and human (HS) extracts as a function of light dose. Reaction mixtures containing 50 fmol of substrate and the indicated amounts of CFE in 50 μl of reaction buffer were exposed to the indicated number of camera flashes. The DNA was then digested with *Mse* I and analyzed by electrophoresis in 12% polyacrylamide gels, followed by autoradiography. (*A*) Autoradiograms of gels. (*B*) Plot of repair kinetics obtained from two experiments, including the one shown in *A*.

Assay with Natural DNA. All of the experiments described so far were carried out with the 20-mer T◇T substrate. However, a negative finding with this substrate may be due to inability of a particular enzyme to bind to such a small oligomer. To address this issue we tested the human, *E. coli*, and rattlesnake CFEs by the transformation assay using UV-irradiated pBR322. Again, efficient repair of DNA was carried out by both *E. coli* and rattlesnake CFEs but none was detectable with human WBC CFE (Fig. 4). The repair rate with *E. coli* extract is consistent with the estimates of enzyme levels of 10 molecules per cell (2) or 5×10^{-6} of total cellular proteins, which repair dimers at a rate of 5–6 T◇T per min per photolyase molecule (14) under the irradiation condition used in Fig. 4. Again, a simple calculation reveals that if humans have photolyase and if it has properties similar to all other photolyases characterized to date, its level cannot be more than 1×10^{-7} of total cellular proteins. Furthermore, mixing of human CFE with rattlesnake CFE did not affect the rate of repair by rattlesnake photolyase, indicating that there is no specific photolyase inhibitor in human CFE and that our failure to detect photolyase in humans is most likely the result of lack of photolyase in humans.

Quality of Extracts. One possible explanation for our failure to detect photolyase in human WBC was that the CFE made from these cells was of poor quality in general and thus defective not only in photolyase but in a host of other enzymes. To address this possibility we tested CFEs from HeLa cells, human WBCs, and rattlesnake muscle cells for a housekeeping enzyme, LDH, and a nuclear enzyme, Pol ε (Table 1). All three cell extract types had comparable levels of LDH, and Pol ε was abundant in actively replicating HeLa cells but at very low (but detectable) levels in the mostly quiescent WBCs and rattlesnake muscle cells. Photolyase was detectable only in rattlesnake muscle cells. We considered the possibility that human WBC CFE might contain inhibitory factors in the crude extract which interfere with photolyase activity and therefore separated the CFE into a low-salt (D1) and a high-salt (D2) fraction by chromatography on a DEAE resin. We failed to detect photolyase activity in either fraction (Table 1).

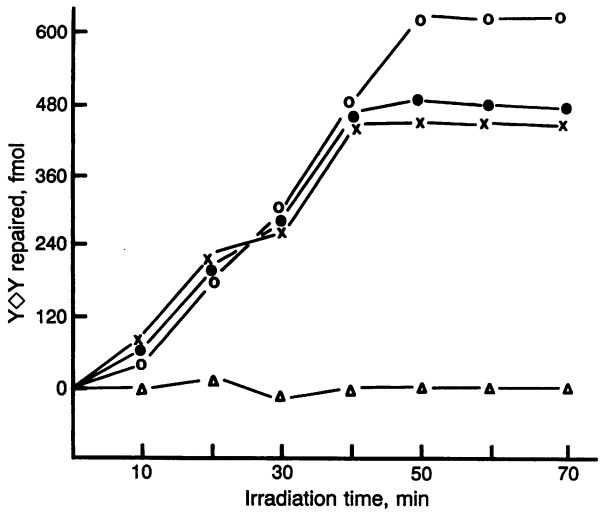

FIG. 4. Repair of Y◇Y in pBR322 by CFEs from *E. coli* (X), rattlesnake (○), rattlesnake muscle plus human WBCs (●), and human WBCs (△). The 50 μl reaction mixtures contained 0.15 pmol of pBR322 (0.8 pmol of lethal photoproducts) and 20 μg of CFE protein. The rattlesnake CFE/human WBC CFE reaction mixtures (●) contained 20 μg of each extract. After irradiation with a black light (λ_{max} 365 nm), the DNA was extracted with phenol/chloroform and transformed into *E. coli* CSR603 to estimate the level of repair. The values are averages from two experiments.

Table 1. Enzyme activities in rattlesnake muscle, HeLa cells, and human WBCs

	Activity, units/mg				
			WBCs		
	HeLa	*C. horridus*	CFE	D1	D2
LDH*	109	400	231	ND	ND
Pol ε†	14	5×10^{-3}	2×10^{-2}	ND	ND
Photolyase‡	<0.3	36	<0.1	<0.2	<0.5

D1 and D2, DEAE low-salt and high-salt fractions, respectively; ND, not determined.
*Unit definition according to ref. 21.
†Unit definition according ref. 20.
‡One unit repairs 1 pmol in 1 hr (6). Values are from data in Fig. 4 for *C. horridus* and WBC CFE. The upper limits for human cells were estimated as follows: 560 μg of WBC CFE, 310 μg of D1, 146 μg of D2, or 220 μg of HeLa CFE showed less than 1/10th the activity of *C. horridus* CFE.

DISCUSSION

Since 1974 a number of reports on human photoreactivating enzyme have been published. It is essential that we critically evaluate some of the key papers and claims on this topic to put the current work in context.

(*i*) Sutherland (6) reported the purification of photolyase to apparent homogeneity in three steps. This protein of 40 kDa was obtained at 24% recovery and had a specific activity of 6350 pmol·mg^{-1}·hr^{-1}. In terms of enzyme kinetics a photolyase molecule with such a specific activity repairs 1 dimer in 4 hr under saturating light. Clearly, such an activity cannot be of physiological significance, and we have been unable to detect even such a low level when the T◇T is measured directly.

(*ii*) Several reports have been published claiming reduced levels or total lack of photolyase activity in XP cell lines (7, 8, 25, 26). From the standpoint of this discussion the most relevant is the paper which reported (7) the following residual activities in XP cell lines (compared to 100% in normal): XP-A, 35.3%; XP-B, 0%; XP-C, 15.8%; XP-D, 8%; XP-E, 49.5%. In recent years XP-A, -B, -C, and -D genes have been cloned and sequenced and in the majority of XP cell lines the mutations inactivating the proteins have been identified and are consistent with inactivation of a subunit of an excision repair enzyme (27, 28). It is difficult to reconcile the reports on defective photoreactivation in XP cells compared to normal cells with the molecular genetics of XP.

(*iii*) Human photolyase reportedly has a nearly flat action spectrum (range of activity within a factor of 3) in the 300- to 600-nm range (29–31). While such a finding in itself does not cast doubt on the authenticity of the activity as that of a photolyase, all known photolyases have well-defined action-spectra peaks and none photoreactivates measurably at wavelengths >500 nm (1, 24, 32, 33).

(*iv*) The first paper on photolyase from humans reported a native molecular mass of 40 kDa for enzyme purified to apparent homogeneity (6). More recently, by using an ultrafilter with a cutoff of 10 kDa, it was found that in all human tissues examined there were at least two molecular species with photolyase activity, one larger than 10 kDa, and one smaller (34). All other photolyases that have been characterized to date have molecular masses of 50–60 kDa and are made up of a single polypeptide with two noncovalently bound chromophores (ref. 24; R. Ley, personal communication), and only a single type of enzyme has been found in organisms ranging from *E. coli* to the marsupial *Monodelphis domestica* (24, 33).

(*v*) The least ambiguous photolyase assay is the chemical assay, which measures the Y◇Y level directly by one of several chromatographic procedures. This assay has been

used in a few studies (6–8). Other studies have used the Norit adsorption assay (34, 35), transformation competition assay (36), and ESS (enzyme sensitive site) assay, which consists of treatment of photoreactivated DNA with T4 endonuclease V followed by separation in alkaline agarose gel (37) or alkaline sucrose gradients (38). More recently immunological methods using antibodies against UV-irradiated DNA (39) or T◇T (40) have been employed. While it is impossible to critically evaluate all these studies here, the following facts are worth mentioning. (*a*) d'Ambrosio *et al.* (38) report that about 500,000 Y◇Y are repaired by photoreactivation in 1 min in a single human skin cell as measured by the ESS assay. It is impossible to reconcile this figure with the report that a human photolyase molecule repairs 1 dimer every 4 hr under conditions of saturating Y◇Y substrate and light (6). In *E. coli* and yeast (2, 24), *in vivo* and *in vitro* experiments yield reasonably consistent results. (*b*) One of the most sensitive assays for photolyase is the transformation assay. Harm (41) easily detected photolyase in marsupial cell extract by this assay but was unable to detect the activity with extract from placental animals. (*c*) Eggset *et al.* (39) used antibodies made against UV-irradiated DNA to measure photoreactivation of Y◇Y in human skin by employing immunohistochemical methods and reported rapid disappearance of antigenic sites when UV irradiation was followed by photoreactivation treatment. However, upon further characterization the authors found that the antigenic determinant for their antibodies was probably the 6-4 photoproduct and not Y◇Y.

(*vi*) It has been reported that in humans, when skin was irradiated with a single UV (254 nm) dose followed by exposure to photoreactivating light, no photoreactivation could be observed; however, when the UV dose was split into three parts administered at 2.5-hr intervals followed by exposure to visible light after each irradiation, the level of antigen reacting with a monoclonal antibody made against T◇T decreased by 40% (40). These results could be interpreted as induction of photolyase by sectored irradiation, followed by splitting of T◇T by the induced photolyase. However, the authors caution that their assay is indirect and does not necessarily mean that this treatment regimen resulted in light-induced monomerization of Y◇Y (42).

(*vii*) It was proposed that a protein which binds to UV-irradiated DNA and is defective in the XP-E complementation group might be the human homolog of photolyase (43). However, later studies revealed that this protein binds to 6-4 photoproducts and not to Y◇Y (44) and therefore cannot be a photolyase homolog.

In the present study we have used a defined substrate and a direct assay for the enzymatic photoreversal of the cyclobutane ring to search for photolyase in human cells. We were unable to detect activity in this system and found that the absence of activity in CFE was not caused by an inhibitor interfering with photolyase. We failed to detect any activity in human WBCs with this assay, which can detect photolyase at a level of 10^{-8} of total cellular proteins, or about 10 molecules per human cell. In summary, a critical evaluation of papers on human photolyase and of data presented in this paper leads us to conclude that it is most unlikely that humans have a DNA photolyase. In agreement with other workers (42, 45), we suggest that any future research on the subject should be conducted with either defined substrate, pure enzyme, or a cloned gene expressed in a heterologous system.

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