An ethenoadenine FAD analog accelerates UV dimer repair by DNA Photolyase

Supplementary Material

Reconstitution of apophotolyase with ε-FAD

ε-FAD was successfully reconstituted following the same protocol as mentioned above but ε-FAD was used in place of FAD[6]. After reconstitution, the absorption spectra shows clear well defined vibronic features as would be expected from a reconstituted flavin. The purity of ε-FAD is not extremely critical for the reconstitution experiments. Apo-photolyase is unable to take in other flavin derivatives such as riboflavin or FMN[7].

Preparation of cyclobutylpyrimidine dimer (CPD) substrate

Two different HPLC-purified DNA sequences were purchased from IDT DNA Technologies having the following sequences:

5-mer: 5'-TTTTT-3'

11-mer: 5'GCAAGTTGGAG-3'

For making CPDs from the 5-mer sequence, a 1.0 mL water solution containing ~150 μL acetone and 5-10 OD of the 5-mer DNA was purged with Ar for 30 minutes on ice in a purgeable sealable quartz cuvette (1.0 cm × 0.4 cm) fitted with a silicone septum. The sealed cuvette was irradiated on ice for 4 hours using two 40 W UV-B lamps at a distance of 10 cm[8]. A flat mirror was place below the cuvette so that the reflected light could double pass the sample. The cuvette was covered with a plastic Petri dish to filter out UV light < 315 nm. After irradiation, the sample was dried using a Savant Speed Vac®. The dried sample was dissolved in water and

purified using RP-HPLC. A YMC-AQ C-18 column and water/acetonitrile mobile phase was used for this purpose. A 7-9% acetonitrile gradient was used to separate the photoproducts. All peaks were collected and dried. Absorption spectra were taken to analyze the ratio of absorbance at 260 nm to 210 nm. When the 5mer sequence is converted to a sequence with one CPD (independent of the location) the A_{260}:A_{210} goes from 0.8 to 0.6. The peak with A_{260}:A_{210} of 0.6 was collected and used in the repair assay experiments.

In the case of the 11-mer sequence, ~50-75 μM of undamaged DNA + 150 μL of acetone + HPLC water totaling to a volume of 1.0 mL was placed in a small pyrex tube (5 cm (L) × 0.7 cm (ID)) and sealed with a rubber septum and parafilm. The sealed tube was purged on ice for 30 min with Ar. The sample was then irradiated using an Oriel 150 W Hg lamp for ~12 min, at a distance of 7.5 inches from the housing. During the irradiation the sample tube was placed inside a glass beaker containing water, which helps filter the shorter UV irradiation. Even though pyrex cuts out shorter UV, it is not sufficient by itself to prevent the formation of other photoproducts. The irradiated sample was then directly loaded on to a C-18 column and purified using an acetonitrile/0.1M triethylammonium acetate (pH=7.0) mobile phase. A 7-11% acetonitrile gradient was used for this purpose. This method is based on earlier reports on thymidine dimer preparation[9].

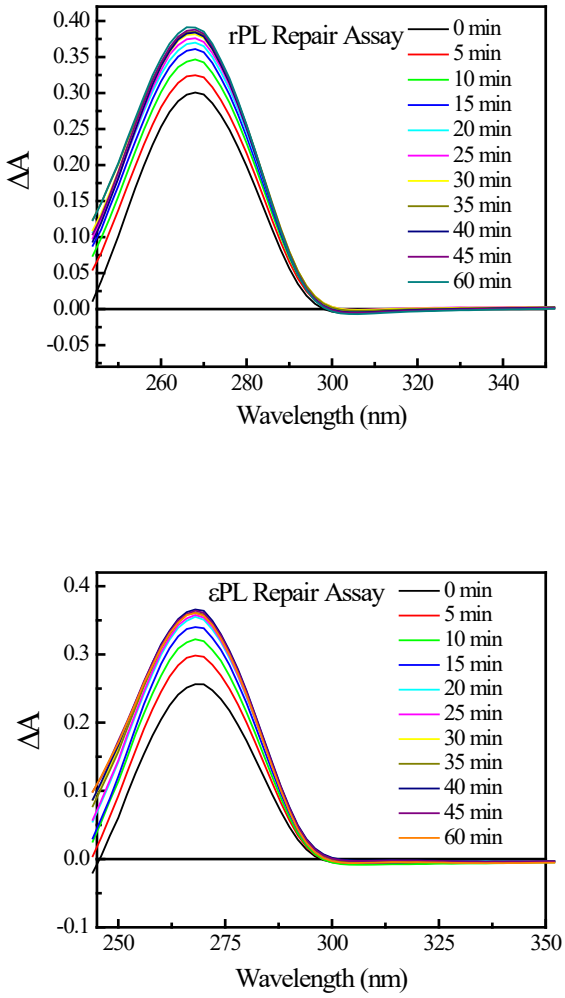

Figure S.1 Typical repair assays of *E. coli* rPL and εPL with a 5-mer substrate containing one T<>T. The difference absorption spectra show repair of CPD by the increasing absorbance of the product T-T at 266 nm. The spectrometer was blanked using the protein sample before the addition of the CPD (0.5 cm path length). The concentration of PL was fixed at 500 nM and the substrate concentration was 10 μM.

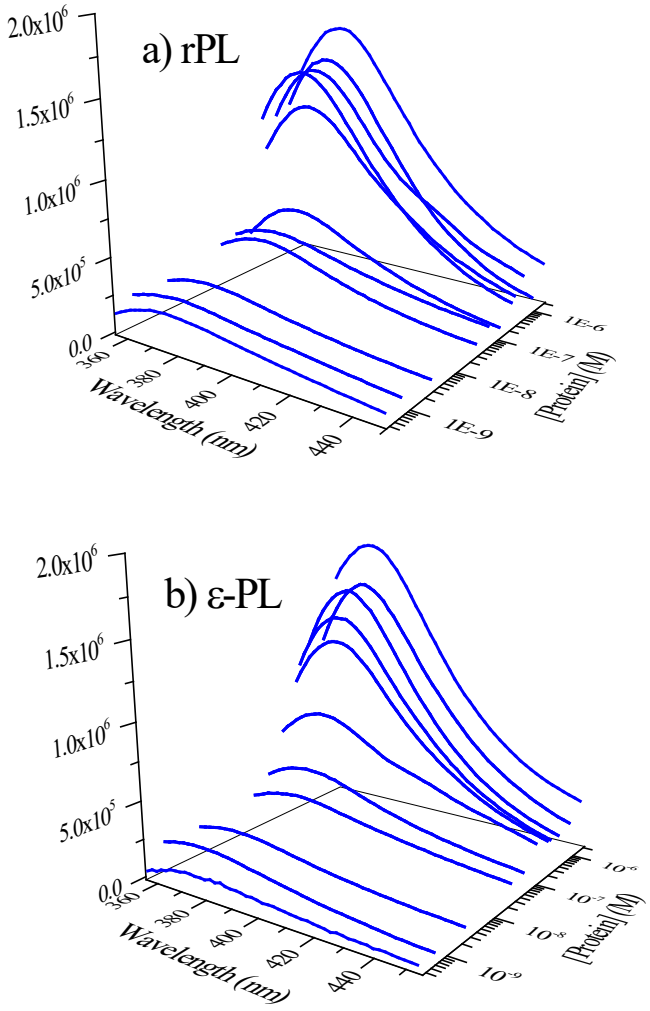

Figure S.2: Plot of fluorescence emission intensity (arb. units) at various concentrations of rPL (top) and εPL (bottom). λ_{ex}=317 nm with emission detected from 335-600 nm. The data shows an increase in fluorescence of 2AP reporter base due to base flipping of the CPD in the presence of increasing concentrations of PL.

References

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