and P_i approaches infinity. The pitch connection then yields $P_{DNA} = N_i\Delta z = 28$ Å for 2.3 nucleotides/subunit. This type of DNA structure, predicted by the pitch connection and five-fold rotational symmetry for fd, might account for the diffraction intensity recently observed on layer spacings of ~26 and 13 Å for magnetically oriented fibers of fd (5).

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PHOTOREACTIVATING ENZYME FROM *ESCHERICHIA COLI*

Interactions with DNA and Mechanism of Action

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In photoreactivation, photochemical damage produced in DNA by far ultraviolet radiation ($\lambda < 320$ nm) is repaired in an enzyme-mediated reaction using longer wavelength light ($310 < \lambda < 450$ nm for *E. coli*). Photoreactivating enzyme (PRE) acts on a single class of photoproducts, cyclobutyl pyrimidine dimers, in an otherwise normal DNA strand at least nine bases long. PRE is one of the few DNA repair enzymes which has been purified to homogeneity in quantities sufficient for physico-chemical studies.

The *E. coli* PRE is a single polypeptide of 32,500 d;[1] it is low in aromatics and appears to lack tryptophan.[1] The protein is associated with an RNA (roughly 10 nucleotides per protein monomer) which is required for activity;[1] its absorption spectrum thus has a peak near 257 nm due to its RNA. The measured spectrum has a trailing optical density at wavelengths > 320 nm. After correcting the measured spectrum for the effects of light scattering (1) we find no true absorption for wavelengths above 320 nm, the spectral region where light must be

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[1]Snapka, R., and B. M. Sutherland. Manuscript submitted for publicaton.

absorbed for enzymatic activity (2). The light-scattering responsible for the apparent optical density above 320 nm results from extensive aggregation of the isolated protein.

When PRE is mixed with UV-irradiated DNA, a new absorption band appears between 300 and 450 nm; there is also a decrease in total absorption below 300 nm (3). The scattering-correction procedure (1) shows that these effects are not due to changes in light scattering, but rather reflect the appearance of a new absorption band above 300 nm and hypochromicity below 300 nm, respectively (3). The new absorption band, which appears to be responsible for the absorption of photoreactivating light, presumably results from interactions of the enzyme and of the pyrimidine dimer or regions of DNA near the dimer. The nature of the interactions of PRE with its substrate are thus of critical importance in the function of the enzyme. Although the PRE is specific for pyrimidine dimers in DNA (it does not bind to dimer-containing RNA), the DNA sites recognized by the PRE must be heterogeneous since all *cis-syn* pyrimidine dimers (T[]T, T[]C, C[]C, T[]U, and U[]U) can be repaired. More than just the dimer is required for binding, since the PRE does not bind to isolated dimers or dimer-containing oligonucleotides less than nine bases long (4). Since the 6 position and the carbonyls at the 2 position of the pyrimidine ring are the only available sites common to T, C, and U, they are likely specific recognition sites (5). The carbonyls at the 2 positions project into the minor groove of the DNA while the saturated 6 positions are in the major groove. We have shown that the peptide-antibiotic netropsin, which binds specifically in the minor groove of DNA, inhibits binding of the PRE (6). Contrary to previous reports, we have shown that netropsin does not greatly alter the helical structure of the DNA to which it binds (7). Netropsin is specific for double-stranded DNA and will not bind directly at a dimer because of the disruption in the helix which a dimer produces (8). It does, however, bind in close proximity to many dimers (8). Netropsin does not interact directly with PRE. Thus the ability of Nt to inhibit binding of the PRE to dimer-containing DNA suggests that the enzyme interacts with moieties in the minor groove.

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