Low-temperature Electron Paramagnetic Resonance Studies Reveal the Structure of Thymine Dimer Radical Anions

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Electron addition at 77 K to *cis-syn* pyrimidine dimers yields the monomer radical anion directly, whereas the *trans-syn* dimer does not split, thus allowing the first observation of the undissociated dimer anion radical species.

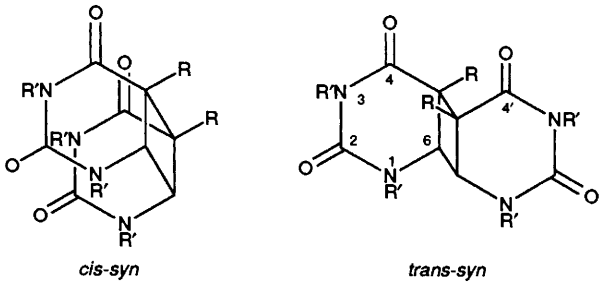
Solar UV (290–400 nm) is known to have mutagenic and carcinogenic effects,[1] a matter of increasing concern due to the depletion of the stratospheric ozone layer. The most common product of UV absorption by double stranded DNA is the *cis-syn* pyrimidine cyclobutane dimer, in addition to small amounts of the *trans-syn* isomer in single stranded DNA.[2]

In a beautiful example of opportunism, nature uses a light induced enzyme system, the photolyases, which utilise the energy of a photon of light (300–500 nm) to monomerise the pyrimidine dimer.[3] Both theoretical and experimental approaches suggest that the photoenzymic pathway proceeds via electron donation to the dimers.[4–6] Hence we have simulated the enzymic process by electron addition to *cis-syn* thymine dimers and both *cis-syn* and *trans-syn* 1,3-dimethyluracil using γ-radiolysis in aqueous lithium chloride glasses[7] at 77 K and used EPR spectroscopy to study the initial electron adducts, and to follow any subsequent reactions using temperature-resolved methods.[7]

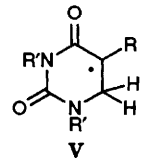
γ-Radiolysis (0.5 Mrads) of 50 mmol dm^{-3} of the *cis-syn* dimers of thymine and 1,3-dimethyluracil (**I**, scheme 1) in 10 mol dm^{-3} LiCl gave the corresponding monomer radical anions (**III**, Scheme 1) directly at 77 K as monitored by EPR spectroscopy (measurements were taken at X-band frequencies). The hypothetical dimer anion intermediate (**II** Scheme 1) was not observed. These monomer anion radical spectra are characterised by well defined anisotropic doublets with isotropic *g*-values close to that of the 'free spin'. The doublets arise from hyperfine coupling to the C–H proton at the C^6 position of the pyrimidine ring. The spectra are identical with those of the radical anions derived directly from the corresponding monomers and generated in a similar matrix.[8]

Previously, it has been shown that the radical ions of thymine and uracil, in such a matrix at low temperature, protonate irreversibly at the C^6 position of the pyrimidine ring upon warming forming a hydrogen adduct. In the case of thymine and its derivatives this species (**V**) gives rise to an octet which completely identifies its structure. Such a protonation is also observed for the *cis-syn* thymine dimer on annealing from 77–150 K and thus provides confirmation of our proposal that the monomer radical anions are indeed formed on irradiation of the *cis-syn* dimer.

In marked contrast, the *trans-syn* isomer of 1,3-dimethyluracil, gives a novel isotropic 19 G EPR doublet [Fig. 1(*a*)] with a *g*-value also close to that of a 'free-spin'. This is lost on annealing above 77 K, with the concomitant growth of the normal 12.5 G doublet characteristic of the monomer radical anion [Fig 1(*b*)]. Since clearly only one proton splitting is observed, we assign the 77 K spectrum to an asymetric dimer



R′ = Me; R = H, 1,3 dimethyluracil
R′ = H; R = Me, thymine



V

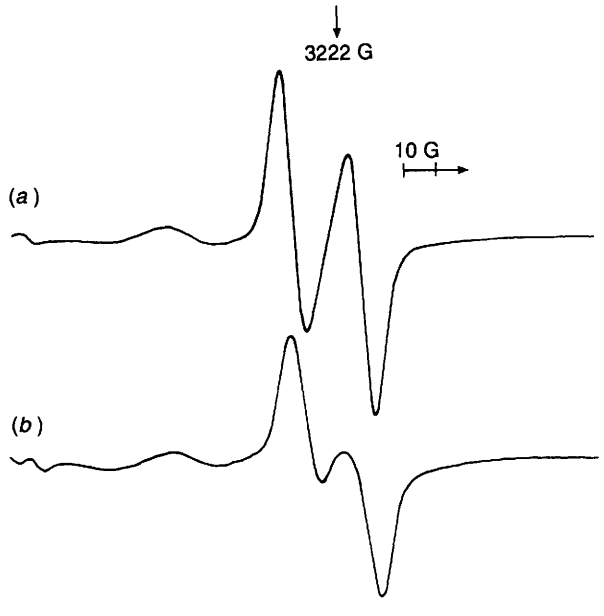
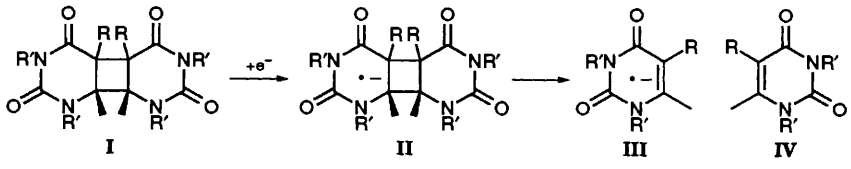


Fig. 1 First-derivative EPR spectra recorded at 77 K for (*trans-syn*) 1,3-dimethyluracil dimer in aqueous 10 mol dm^{-3} lithium chloride after exposure to ionising radiation at 77 K, (*a*) showing features assigned to the dimer radical anion and, (*b*) after annealing to 135 K, showing features assigned to the monomer radical anion



Scheme 1 Splitting of pyrimidine dimers following electron addition

radical anion where the large hyperfine coupling must stem from σ–π overlap with the β-proton (at C^5), as shown pictorially in Fig. 2.

Whilst the spin-density on the adjoining ring carbonyl carbon atom $C^{4'}$ and the neighbouring nitrogen atoms (N^1 and N^3) must be low. This postulate is supported by previous EPR measurements on dihydrouracil[10] and dihydrothymine.[11]

We tentatively suggest that the driving force for dissociation is the partial occupancy of the C^5–$C^{5'}$ σ* orbital in **2** resulting in a weakening of this bond. This in turn leads us to postulate that the two bonds may break consecutively rather than in a concerted manner. In fact, the conservation of orbital symmetry forbids splitting of the cyclobutane ring by a thermal concerted mechanism. Electron addition to Pyr<>Pyr is predicted to decrease the energy barrier to a concerted non-synchronous or completely non-concerted splitting of the dimer anion radical $(Pyr<>Pyr)^{\cdot -}$ causing an initial cleavage of the C^5–$C^{5'}$ bond of the cyclobutane ring.[12]

In addition, the results establish that the radical anions of the *cis*-isomers are less stable than those of the *trans*-isomers. If a small increase in strain enhances the splitting rate then this has important biological implications as the *cis-syn* dimer in DNA has been shown to be puckered due to the deformation of the double helix.[13] Earlier studies of photosensitised splitting also found that the stereochemistry of the dimers was important in determining the splitting rate,[14] but it was not known at the time whether the initial electron addition process or the subsequent cleavage of the cyclobutane ring was effected. In fact steric repulsion between the two pyrimidine rings will be greater in the *cis* isomers, an interaction further enhanced by the added electron in the radical anion species.

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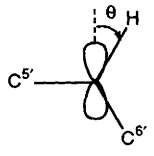

Fig. 2 View along the C^4–C^5 axis of the *trans-syn* isomer showing that the σ–π overlap between the C^4 p orbital and C^5 proton is dependent on the angle θ. The hyperfine splitting of the proton is proportional to $\cos^2\theta$.

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