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Physical Model of Photoreactivation

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1. Introduction

Ultraviolet light in the range of 220 nm to 320 nm has lethal, mutagenic and carcinogenic effects on the biological systems [1]. Killing and mutation of bacterial cells by ultraviolet light is a useful model of these effects. Bactericidal action spectra matched more closely the absorption spectra of nucleic acids than those of proteins, indicating that the lethal effect is due to specific nucleic acid damage [2]. The action spectrum for the induction of mutations resembles the bactericidal action spectrum [3, 4].

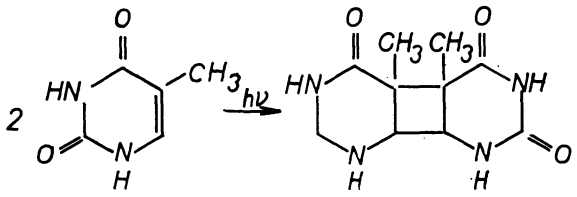
Genetic information of bacteria is condensed into a single deoxyribonucleic acid molecule (*DNA*). The structure of *DNA* is well-known — it is a Watson-Crick double-stranded helical polymer [5]. Each strand has a sugar-phosphate backbone with one base attached to each sugar. There are four bases: two pyrimidine derivatives — thymine and cytosine, and two purine derivatives — adenine and guanine. Each base from one strand is hydrogen-bonded to another coplanar base which is attached to the other strand. The planes of the bases are perpendicular to the helical axis, the distance between planes is 3.36 Å.

Exposure to ultraviolet light (UV) produces various kinds of stable photoproducts in *DNA*. Among these products thymine dimers ($\widehat{TT}$) represent the main biological lesions [6]. Dimers caused 50 to 90% of the lethal effect of UV in the cell [7]. It has long been known that irradiated bacterial cells show different survival levels depending on various postirradiation enviromental factors. This phenomenon was interpreted by many authors as recovery, repair, reactivation. Photoreactivation – a repair which occurs in cells held in visible light — generally involves the enzyme photosensitized monomerization of pyrimidine dimers in the *DNA* [25].

There are two broad areas of photobiology in which the excitation energy transfer may be of interest to the International Seminar. The first deals with the induction of thymine dimers in *DNA* by UV. The second area is concerned with the physical mechanism of photoreactivation. The plan of this work is to preserve the biological emphasis and to stress those topics which appear to be most relevant for these biological applications. Attention will be concentrated mainly to thymine and thymine dimers.

2. Formation of Thymine Dimers

The dimerization of thymine is the cycloaddition of two thymines at the 5.6 double bond to give dimer, which is a cyclobutane derivative [8]:



(1)

Theoretically, four conformations of final product are possible [9]; they are shown in Fig. 1. In general, all four configurational isomers are formed in solution [10]. In *DNA* as well as in frozen aqueous solution of thymine only the cis-syn dimer was found [11, 12].

Thymine undergoes photodimerization under a variety of conditions. At sufficiently low monomer concentrations ($\lesssim 10^{-3}$ M) at room temperature, photodimers can be formed only as a result of the interaction of a ground-state monomer with a triplet-state molecule. This is so because collision between a ground-state molecule and an excited singlet-state thymine is precluded because of the very short life-time of the latter [13] — solute molecules cannot diffuse together in order to dimerize within the singlet lifetime 10^{-12} sec. Johns and his group were able to make use of the simple scheme ilustrated in Fig. 2 to interpret their kinetic studies of the photoreactivation of thymine in dilute solution as a function of the thymine concentration and the concentration of oxygen [14, 15]. Dimer quantum yield in this system depends on the wavelength of the exciting light; a greater yield was obtained at the shorter wavelengths. The effect was attributed to a wavelength-dependent intersystem crossing ("more triplets being formed at the shorter wavelengths"). The wavelength effect begins at the red edge of the

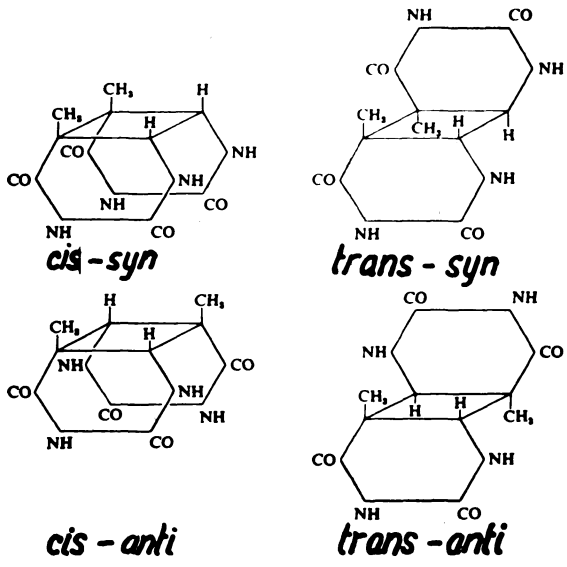


Fig. 1. The molecular structures of the four cis-fused cyclo butane dimers of thymine

lowest absorption band of the thymine. In the presence of such a wavelength effect, the precursor state is likely to be an excited triplet state. Triplet state has been detected by flash photolysis technique and has been characterized by its spectrum. Triplet quenching experiments have shown that triplet is the precursor state in dilute solutions [16].

As the concentration of the thymine is increased, an increase in the rate of $\widehat{TT}$ formation occurs when ground-state association ("stacking") begins. Wierzchowski and his group have shown that in the high-concentration range triplet quenchers are ineffective in quenching the dimerization in water; dimerization in organic solvents (= prevention of stacking interaction) can be partially quenched by triplet quenchers, but the unquenched portion has a different isomer ratio [17]. These results indicate that different paths exist for dimerization in aggregates and in dilute solutions. Fig. 3 shows dimerization in concentrated solutions where $\widehat{TT}$ formationproceeds either directly from a singlet state or by way of a tri plet state.

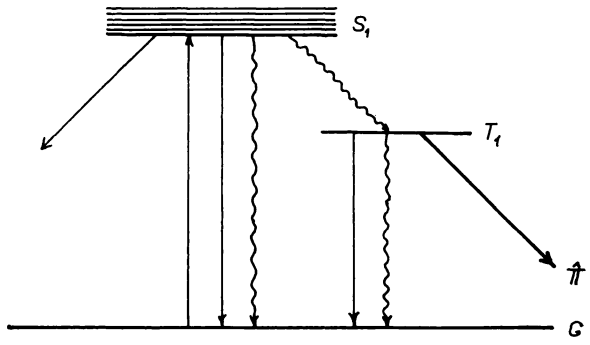


Fig. 2. A model for the kinetics of thymine dimer formation in aqueous solution. G, S_1 and T_1 are the ground state and lowest excited singlet and triplet states, respectively

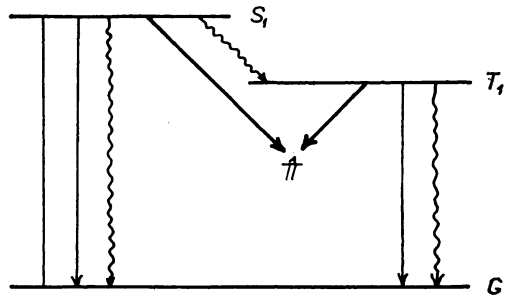


Fig. 3. Thymine dimer formation in concentrated aqueous solutions

The dimer yield in dilute solutions is 10^{-3}, it is $4 \cdot 10^{-2}$ in concentrated solutions, but it is $\sim 10^{\circ}$ in frozen water solution of thymine [6]. The reason for the high efficiency of dimerization in ice is that, in freezing, microcrystals of thymine hydrate are formed in which neighbouring thymines are parallel and suitably placed for dimerization [18]. Eisinger and his group obtained a clear picture of thymine dimerization in microcrystals in their studies on oriented monomer pairs [13]. They dissolved pure dimers in ethylene-glycol-water and cooled the sample to 80°K, where a clear glass is formed. Dimers were split into a pairs of monomers with 248 nm UV. The broken dimers redimerize with a quantum yield of 1.0 ± 0.1. The simplest interpretation of the results for thymine crystals and broken dimers is that the excited

singlet goes on to form dimer faster than anything else. An alternative pathway would involve ~ 100% intersystem crossing from the excited monomer pair followed by ~ 100% efficient dimerization from the triplet state. Eisinger has found that the absorption spectra of broken dimers exhibit exciton splitting [19]. Excitation of the lower exciton state leads to the dimer with the unity quantum yield. The lower exciton state should be smoothly connected with the excimer (excited dimer) state, so that the latter is most likely an intermediate in the photodimerization in broken dimers. Dimerization in thymine crystals is schematically shown in Fig. 4.

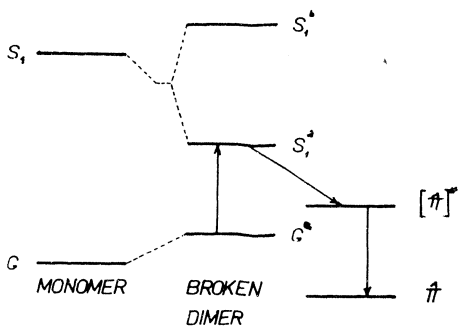


Fig. 4. The energy levels of the thymine monomer and the "broken dimer". G^e, S_1^a and S_1^b are the ground state and the lowest excited singlet states after excitonic splitting. Photodimerization proceeds by way of the excimer state $[\widehat{TT}]^*$

The *DNA* molecule is a very complex system. The Watson-Crick structure provides for a 36° angle between neighbouring thymines, whose molecular planes are spaced 3.36 Å apart. Thus, neighbouring thymines in *DNA* are neither like thymine in dilute solution nor like thymines in ice. The quantum yield for dimers in DNA of about 10^{-2} is 10^2-times smaller than the yield in ice; it is four-times smaller than in concentrated solutions, but 10-times larger than the yield in dilute solutions [6]. The addition of triplet quenchers does not affect the rate of dimer production in *DNA* [20, 21]. This is consistent with a model in which dimerization is a singlet-state process. On the other hand, photodimerization in DNA can occur by way of the triplet state since the reaction can be sensitized with triplet donors; this includes the population of the triplet state of thymine in *DNA* from the triplet state of a suitable sensitizer [22]. It is probable that dimers in *DNA* are formed directly from the excited siglet state as well as via the triplet state, with triplets arising from intersystem crossing from higher vibrational levels of the first excited singlet.

It is important to note that *DNA* phosphorescence at 80°K is not the sum of the emission from individual nucleotides [23]. *DNA* emission was identified to be the thymine triplet emission. Evidence is available from electron spin resonance to show that the triplet state of *DNA* has propetires of thymidine triplet state [24]. It follows from this that excitation energy must be transferred preferentially to the thymines, and this may be the reason why $\widehat{TT}$ are the main photochemical lesions in *DNA*.

3. Monomerization of Thymine Dimers

Monomerization or "splitting" of thymine dimers to the monomers — two thymines — is regarded as the chemical reaction involved in the photoreactivation process.

It is well-known that, upon excitation to an electronic level by the absorption of a short UV wavelength, $\widehat{TT}$ are split to the monomers very efficiently [6]. The quantum yield for this reaction in water solution and at room temperature vary between 0.6 and 0.9 over the wavelength range of 200–289 nm [26]. The quantum yield for $\widehat{TT}$ monomerization in ethylene-glycol-water glass at 80°K by 248 nm is approximately unity [19]. This very high quantum yield for the $\widehat{TT}$ monomerization by UV is consistent with the model in which monomerization is the excited singlet state reaction.

On the other hand, $\widehat{TT}$ can be split via the radical reaction mechanism. As early as 1960, Beukers and Berends noted that mass spectroscopy gave an anomalous molecular weight of 126 for thymine dimer; they postulated a breakdown of the $\widehat{TT}$ under the duress of electron bombardment [8]. Jennings and coworkers observed that none of the mass spectra of the dimers show the dimer parent peaks [27]. Evidently, after ionization, the dimers collapse quantitatively to thymines and ionized thymines, so that monomerization must occur after ionization. Jennings suggested the following reaction scheme for this process:

$$e + \widehat{TT} \longrightarrow \widehat{TT}^{+\circ} + 2e \longrightarrow T + T^{+\circ} + 2e \longrightarrow \qquad (2)$$

Furthermore, when aqueous solutions of thymine dimers are subjected to ionizing radiation it was found that the main radiolysis product was thymine [28, 29]. Deering and Snipes [30] re-investigated monomerization of $\widehat{TT}$ under ionizing radiation and suggested this reaction scheme:

$$\widehat{TT} + h\nu \longrightarrow \widehat{TT}^{\circ} \rightarrow T^{\circ} + T \qquad (3)$$

They found that $\widehat{TT}$ breakage by UV light produced by gamma-rays in the $\widehat{TT}$ solution from Čerenkov radiation and from excited water molecules was insignificant in the radiolysis of $\widehat{TT}$ solution.

Weinblum reported the splitting of crystalline dimethylthymine dimers ($\widehat{DMTDMT}$) to dimethylthymines (DMT) by gamma-irradiation [31]. The cis-syn and trans-syn isomers are split very efficiently with initial G-values of 6300 and 1700 respectively. These high values can only be explained by a chain reaction. Splitting of two-strained cyclobutane single bonds and subsequent formation of two carbon-carbon double bonds require about 0.6 eV. The net energy gain of the splitting reaction is then of the order of 2–3 eV (2-times the mesomeric resonance energy of DMT minus 0.6 eV). Splitting is an energy-delivering reaction. Therefore, chain reaction is possible. The first possible mechanism of transfer of activation energy through a crystal might be exciton transfer – if ionizing radiation could split dimers via an electronically excited state. If this is true, and if ionizing radiation excite singlet state, then the same chain reaction should be initiated by UV in the crystalline dimer. However, it was found that UV at 240 nm does not initiate a chain

reaction in this case [32]. From this follows that the first excited state is not the chain initiating species, but most likely a more energetic or ionized state. The second possibility is very intriguing, because the only particle which can diffuse through a crystal in a short time is an electron. One could assume then that primarily an unstable anion of dimer is formed and subsequently disintegrates into *DMT* and *DMT* anion. The latter may be able to transfer its additional electron to a neighbouring dimer to form dimer anion which again disintegrates. Therefore, the reaction scheme can be described as follows:

$$\widehat{DMTDMT} + e^- \longrightarrow DMT + DMT^- \dashrightarrow 2\,DMT + e^- \qquad (4)$$

One can equally assume a dimer cation as the chain initiating species.

Results recently obtained in similar compounds support the hypothesis as to participation of an electron in the splitting reaction. Namiki and Hayashi have found that radiation-induced thymine formation from thymine glycol is inhibited by N_2O – a very efficient scavenger for solvated electrons [33]. Snipes and Bernhard observed formation of thymine in the irradiated dihydrothymine solution [34]. Dihydrothymines react with solvated electrons, yielding an anion radical [35].

It is thus evident that $\widehat{TT}$ can monomerize via two distinct reaction mechanisms – either by way of an excited singlet state or by way of a radical reaction mechanism. The first step in the radical reaction is probably formation of an anion radical.

4. Photosensitized Monomerization of Thymine Dimers

The monomerization of dimers by the use of chemical photosensitizers might serve as a most useful model for studying the mechanism involved in photoreactivation [37–40]. Photosensitized splitting of thymine dimers by indole derivatives may throw some light on this problem.

Reflectance and luminiscence measurements were used to show that tryptophan forms intermolecular complexes with the pyrimidine nucleosides in frozen aqueous solutions [41]. A new absorption band appears at wavelengths longer than the absorption bands of the components, and besides there is a new fluorescence band characteristic of the complex. Formation of complexes in fluid aqueous solutions was observed in proton magnetic resonance, and absorption and circular dichroism studies [42, 43, 44]. Indole derivatives likewise form intermolecular complexes with pyrimidine dimers in fluid aqueous solutions as well as in frozen aqueous solutions, as shown by proton magnetic resonance experiments [45].

Indole derivatives are known to be good electron donors; they form electron donor-acceptor complexes with several electron acceptors [46]. Indole fluorescence is quenched by a series of electron scavengers; the degree of quenching is related to the electron affinity of the scavenger [47]. Fluorescence is quenched upon interaction with pyrimidine derivatives [44]. Thymine dimers also quench fluorescence of indole derivatives [40].

Thus, it may be concluded from the results shortly rewieved above that indole derivatives (including tryptophan) and pyrimidine derivatives (including $\widehat{TT}$) in aqueous solutions form intermolecular complexes. In aqueous media, several complexations of biological interest are known which can well be explained by the occurence of charge-transfer between reacting species [48]. The appearence of a new absorption is characteristic of weak charge-transfer complexes [49]. In the ground state, the stabilizing forces are mainly van der Waals-London electrostatic forces. Electron occurs to be transferred upon excitation; charge-transfer contribution is thus enhanced in the excited state of the complex. The quenching of fluorescence observed in the complexes in frozen aqueous solutions has frequently been ascribed to this electron transfer. Such an electron transfer probably occurs also in the complexes forming in fluid solutions. Thus, reactions between thymine dimer and tryptophan (*trp*) can be described by the following reaction scheme:

$$trp + \widehat{TT} \rightleftharpoons [trp^{\delta+}\ \widehat{TT}^{\delta-}] \xrightarrow{h\nu} [trp^{\delta-}\ TT^{\delta-}]^{*} \longrightarrow trp^{+} + TT^{-} \qquad (5)$$

where $[trp^{\delta+}\ \widehat{TT}^{\delta-}]$ and $[trp^{\delta+}\ \widehat{TT}^{\delta-}]^{*}$ are intermolecular complexes in ground and exited state respectively. According to reaction scheme (4) anion of dimer might disintegrate and two thymines might form.

Helene and Charlier have recently observed such a reaction [40]. Pyrimidinedimers are split in the presence of tryptophan and 5-hydroxytryptophan in aqueous solutions by irradiation at wavelengths where only the indole derivatives absorb light ($\lambda > 300$ nm). This reaction is more efficient in frozen than in fluid solution. Splitting of dimers can be ascribed to the electron transfer from the tryptophan to the dimer in the excited state of the intermolecular charge-transfer complex. If the reactions (4) and (5) are true, one cannot fail to observe photosensitized formation of thymine dimer radicals in the $\widehat{TT}$ + trp frozen solution.

In cooperation with Dr. O. A. Azizova (Institute of Biological Physics, Academy of Sciences, USSR, Puščino-

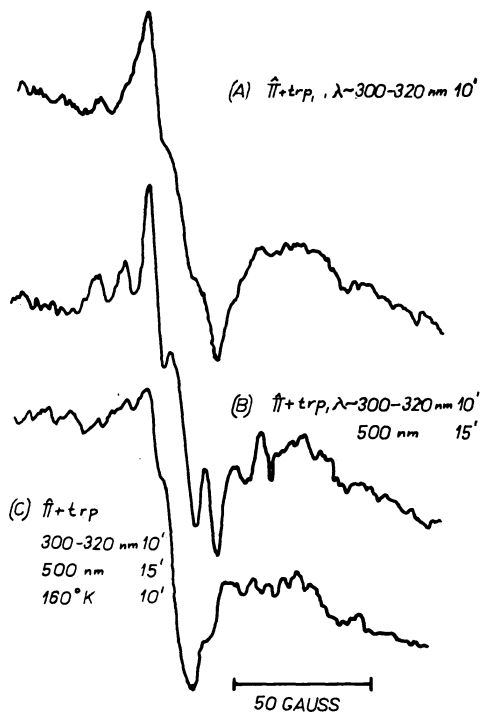


Fig. 5. First derivative ESR spectra of a 5 . 10⁻³ M $\widehat{TT}$ solution in H_2O in the absence (a) and presence (b, c) of a tryptophan (5 . 10⁻³ M) after UV-irradiation. The arrows indicate the lines of the 5-thymyl radical. Magnetic field increases to the right

na-Oke) I have tried to detect free radicals in this system. Tryptophan photosensitizes formation of various light- and temperature-sensitive radicals in the cis-syn thymine dimer in frozen aqueous solution at 77°K. Typical electron spin resonance spectra are shown in Fig. 5. Irradiation with 280–380 nm and corresponding measurements were carried out at 77°K. There is no radical formation in the tryptophan aqueous solution ($5 \cdot 10^{-3}$ M) in our conditions of irradiation. Small resonance appears in the $\widehat{TT}$ solution ($5 \cdot 10^{-3}$ M). This resonance is photosensitized by tryptophan in the $\widehat{TT} + trp$ aqueous mixture ($5 \cdot 10^{-3}$ M each). The resonance splitting with the observed intensity distribution indicates 5,6 dihydro-5--thymyl radical [50]. Snipes and Bernhard have found that the 5-thymyl radical forms in the gamma-irradiated thymine dimer and in the dihydrothymine [34]. Similar radicals were recently reported for dihydro-6-methyl-uracil gamma-irradi-ated at 294°K [51]. The spectral parameters for these radicals are similar.

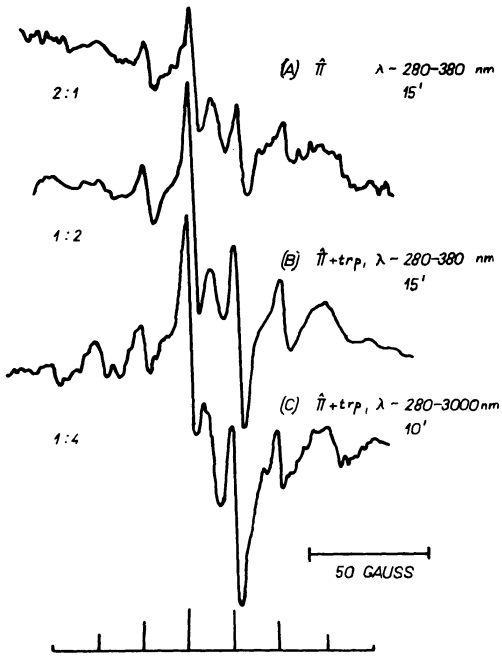


Fig. 6. ESR spectra (first derivatives) of $\widehat{TT} + trp$ aqueous solution (concentration 0,5 M each) (a) Initial spectra immediately after 300–320 nm irradiation. (b) Spectra after photobleaching with $\lambda > 500$ nm. (c) Final spectra after warming to 160° K for 10 minutes. Magnetic field increases to the right

Alternatively, another reaction scheme for the thymine dimer monomerization can be suggested. UV irradiation of tryptophan leads to electron ejection. Formation of the cation radical of tryptophan occurs after two-quantum absorption; the intermediate level is most likely the triplet state [52]. In frozen state free solvated electrons are trapped and absorption of electrons can be observed ($\lambda \sim 600$ nm). Solvated electrons may react with the dimer on warming (or after photobleaching).

If correct, the following hypothetical reaction scheme would satisfactorily explain the various results presented in this chapter:

$$trp + \widehat{TT} \rightleftharpoons [trp^{\delta+}\ \widehat{TT}^{\delta-}] \xrightarrow{h\nu} [trp^{\delta+}\ \widehat{TT}^{\delta-}]^* \longrightarrow trp^+ + \widehat{TT} \qquad (6A)$$

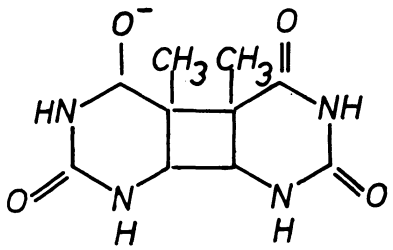
$$\hookrightarrow trp^+ + [\widehat{TT}^-]\cdot \longrightarrow \cdots \longrightarrow 2T + trp$$

$$trp + \widehat{TT} \xrightarrow{h\nu} trp^+ + e^- + \widehat{TT} \longrightarrow trp^+ + \widehat{TT}^- \qquad (6B)$$

Part A of this reaction is a predominant process in frozen state because, upon freezing, the formation of ice crystals forces solute molecules into solid aggregates (and complexes). In fluid solutions hydrated electrons ejected from tryptophan might react with the dimers and in this case part B predominates although not necessarily alone; there also complexation occurs.

UV flash photolysis experiments suggest that hydrated electrons induce dimer splitting in the fluid tryptophan-dimer aqueous system (C. HELENE, personal communication); transient absorption spectra obtained during 35 MeV electron 1 μsec pulse radiolysis support the suggested reaction scheme [53]. Formation of the thymine dimer anion is common for both parts of the reaction scheme. The above discussion indicates that the electron addition to the thymine dimer is an intermediary step in the radical reaction leading to monomerization of the thymine dimer.

Therefore, I have irradiated $\widehat{TT}$ + *trp* system with 300–320 nm. After irradiation, I have photobleached this sample by visible light ($\lambda > 500$ nm). Resonance of the 5-thymyl radical appears on photobleaching. When this sample was subsequently warmed to 160°K, the 5-thymyl radical disappeared. Spectra obtained in these experiments are shown in Fig. 6. Although I have not been able to assign any signal to $\widehat{TT}$. or $\widehat{TT}^{\cdot-}$, its role in the formation of the 5-thymyl radical and in monomerization reaction cannot be ruled out. It is possible to suggest the structure of $\widehat{TT}^{-}$ to be analogous to that of $DHT^{\cdot-}$: dihydrothymine anion radical [35]:



(7)

This anion radical is an hypothetical initial product which later converts into a protonated species. However, details of the subsequent reactions are not known.

5. Physical Model of Photoreactivation

The enzyme-sensitized photoreactivation process, which leads to the repair of the major part of the photochemical lesions in DNA, has received much attention. Its enzymatical mechanism was studied in details by Rupert and the two Harms [54]. They have found that the enzyme molecule E attaches to the DNA region occupied by dimer, which serves as its substrate S, by a light-independent reaction to form en enzyme-substrate complex ES; a subsequent photochemical reaction in the complex restores the pyrimidine ring structure P, releasing the enzyme:

$$E + S \rightleftharpoons ES \xrightarrow{h\nu} E + P \tag{8}$$

The action spectrum for this reaction is in the range from 320 nm to 500 nm, maximal effect occuring in the 355–385 nm region.

Possible mechanisms for this process were suggested [38]: photosensitized splitting of the pyrimidine dimer through an energy transfer process [reaction scheme (9)] and/or formation of a complex and transfer of energy in this complex after excitation [reaction scheme (10)]:

$$E \xrightarrow{h\nu} E^* \qquad E^* + S \longrightarrow E + S^* \qquad S^* \longrightarrow P \tag{9}$$

$$E + S \longrightarrow ES \qquad ES \xrightarrow{h\nu} [ES]^* \qquad [ES]^* \longrightarrow P + E \tag{10}$$

Reaction (9) can be excluded on the basis of the abovementioned enzymatical data [54]. A detailed physical mechanism of the reaction (10) was not presented by the authors cited; it thus remains a mystery for the time being. To solve this problem it is necessary to know the absorption spectrum of the pure enzyme. In the purest preparations so far reported in the literature, Muhammed has not found any absorption maximum in the photoreactivation region [55]. Werbin was able to observe the absorption spectra of the enzyme from baker's yeast with a maximum at 380 nm [56] and from the algae *Anacystis nidulans* with a maximum at 418 nm [57]. Eker's enzyme from baker's yeast exhibites a marked absorption spectrum with a protein peak at 273 nm and a broad absorption band with maxima at 428 and 446 nm [58]. Sutherlands reported strong absorption at 300 nm for the *Escherichia coli* enzyme [59]. In unpublished work, J. K. Setlow from Oak Ridge Laboratories, USA, has achieved a roughly 10^6-fold purification. The resulting highly labile preparation approaches pure enzyme, and the observation that this preparation had no optical absorption at the photoreactivating wavelengths thus seemst o be significant and of very great importance [60].

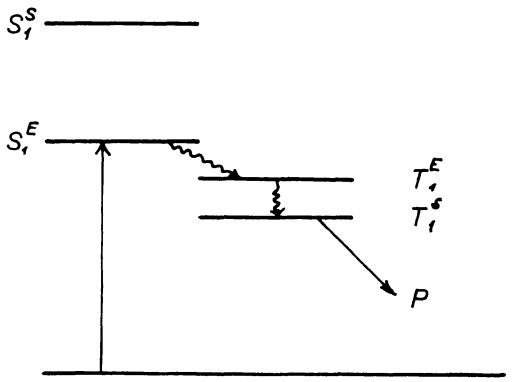


Fig. 7. Photoreactivation as a triplet-triplet energy transfer between excited triplet state T_1^E of the enzyme and the triplet state T_1^S of the substrate after intersystem crossing ($S_1^E \rightarrow T_1^E$) in the enzyme. P is the repaired product

Provided that the enzyme absorbs in the 300–500 nm region excitation energy must be transferred between triplet levels as shown in Fig. 7, because dimer absorbs in the 220–280 nm region [6]. Since Harm reported quantum yield for the photoreactivation reaction between 0.1 and 1.0 [61], a possible role of a triplet — triplet transfer in this reaction may be discounted — it would involve very efficient intersystem crossing in the complex. Two quantum excitation can be excluded — apparently only one photon is required because, unless saturating intensities are reach-

ed, the amount of photoreactivation increases linearly with the total number of photons and is independent of the light intensity [7].

It is possible that enzyme-mediated, light-dependent splitting of pyrimidine dimers is an electron transfer reaction. The photoreactivating enzyme forms complexes with UV-irradiated *DNA*. These complexes – but not the free enzyme – absorb photoreactivating light. Electron might be transferred from the excited state of the *ES* complex to the dimer. The dimer anion is then monomerized in the free radical reaction and the resulting electron comes back to the enzyme cation. Let us suppose that photoreactivation follows this reaction scheme:

$$E + S \rightleftharpoons [E^{\delta+} S^{\delta-}] \rightarrow [E^{\delta+} S^{\delta-}]^* \rightarrow E^+ + S$$
$$\rightarrow E^+ + e + P \rightarrow E + P \qquad (11)$$

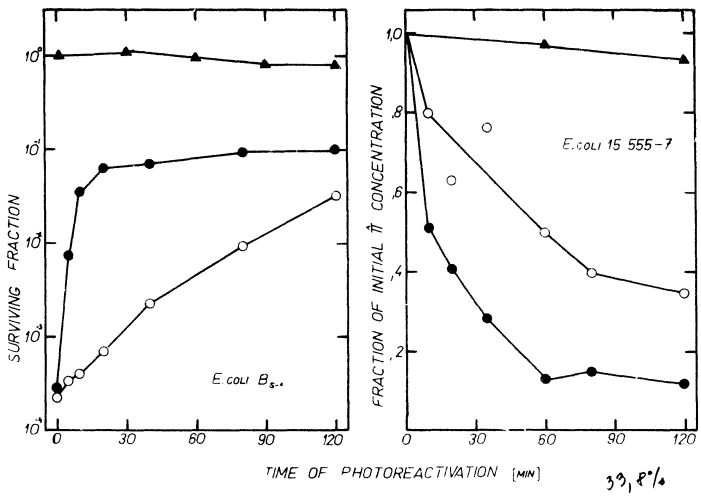


Fig. 8. Effect of KNO_3 on the survival increase of E. coli B_{s-1} cells and on $\hat{TT}$ monomerization in E. coli 15 555-7 as a function of photoreactivating light in the presence ⊖—⊖—⊖ and in the absence ●—●—● of 0,5M KNO_3. Effect of 0,5M KNO_3 on the cells held in dark is also shown ▲—▲.

This may be true if the photoreactivation is inhibited by electron scavengers. I have found that KNO_3, which is a very efficient electron scavenger, a highly efficient quencher of indole fluorescence and inhibitor of a possible electron transfer to pyrimidines [47, 62], inhibits the rate of photoreactivation and monomerization of thymine dimers during photoreactivation of UV-irradiated *Escherichia coli cells* (Fig. 8).

On a theoretical basis, Yomosa has suggested that the enzyme-substrate complexes may generally be charge-transfer complexes formed in aqueous media of living systems [63]. Photoreactivation seems to be such a charge-transfer reaction in the dark step followed by the electron transfer reaction in the photolytic step.

6. Conclusion

The results that I have presented show that photosensitized splitting of thymine dimers and photoreactivation are charge-transfer and electron-transfer reactions. Photoreactivation has been demonstrated in a variety of plant and microbial cells; it is almost ubiquitously demonstrated throughout the animal kingdom. A major exception to this generalization is seen in the placental mammals (including men). where photoreactivation is absent [64]. Thus, photosensitized removal of pyrimidine dimers could be of importance in human cells which also lack another repair mechanism the dark repair mechanism. It has been shown that skin cells from patients with the disease *Xeroderma pigmentosum* do not perform excision repair (i. e. dark repair) of UV-induced damage to their *DNA* [65]. *Xeroderma pigmentosum* is characterized by extreme sensitivity to sunlight resulting in changes in skin cells which eventually lead to multiple actinic carcinomas [66]. The use of photosensitized reactions to remove the dimers could provide a convenient way to overcome this type of cell deficiency.

However, I hope that it is clear from this report that a number of fundamental questions in this field are still unanswered.

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