Influence of Environment on the Photooxidation of Tryptophan with the Carcinogenic Hydrocarbon 3,4-Benzopyrene in Aqueous Solutions of Soap, Caffeine, and Urea

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3,4-Benzopyrene, tryptophan, photooxidation

The solvent dependence of the photooxidation of tryptophan and 3,4-benzopyrene in aqueous solutions was studied by quantum yield measurements. When the hydrocarbon is dissolved in aqueous solution of caffeine, the quantum yields indicate a 3,4-benzopyrene photosensitized tryptophan oxidation instead of a photocooxidation, which is indicated in aqueous solution of sodium dodecylsulfate. The same photosensitized oxidation as in caffeine solution is observed, when urea (6 M) is added to the soap solution, while the fluorescence and absorption spectra indicate no change in the solvation state of the hydrocarbon, comparable to the change from hydrophobic solubilization by the detergent to dipole — induced dipole complex solubilization by caffeine. It is concluded that the difference in the reaction pathways is caused by different solvation states of the excited or reacting oxygen. In the discussion of the results it is referred to reactions of inhibitors.

Introduction

It has been shown earlier [1, 2], that tryptophan is photocooxidized with 3,4-benzopyrene in aqueous soap solutions, when the solutions are irradiated in the long wavelength UV-absorption range of the hydrocarbon. In this paper it is studied, how the reaction pathway depends on the aqueous solvent system.

The changes of the composition during the photoreactions are observed in the absorption spectra of the solutions. From their evaluation together with measurements of intensities of the incident and the absorbed light the quantum yields of the reactions are calculated. The quantum yields differ for the photocooxidation reaction

$$\mathrm{Bp} + \mathrm{Tr} \xrightarrow[\mathrm{O_2}]{h\nu} \text{Bp-, Tr-, Bp-Tr-photooxidation products}$$

(Bp = 3,4-benzopyrene, Tr = tryptophan)
and the sensitized tryptophan-photooxidation

$$\mathrm{Bp} + \mathrm{Tr} \xrightarrow[\mathrm{O_2}]{h\nu} \mathrm{Bp} + \text{Tr-photooxidation products},$$

where 3,4-benzopyrene acts as a sensitizer, but is not changed itself. It is shown that the solvent influence determines whether a photocooxidation or a photosensitized oxidation takes place. Moreover, together with the spectral characteristics in absorption and fluorescence, which are influenced by the solvation state of the hydrocarbon, the evaluation of quantum yields shows what kind of an environmental change is active to shift the reaction pathway from the photocooxidation to the sensitized tryptophan photooxidation.

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The solubility of polycyclic aromatic hydrocarbons in aqueous solutions of detergents was first shown by Ekwall *et al.* [3]. It is due to hydrophobic areas of the micelles, which are formed by the detergent molecules. According to Frank and Evans [4], Stauff [5], Kauzmann [6], and Némethy and Scheraga [7] in the interface of hydrophobic ranges and water, water structure has a higher degree of order than water structure in pure water. Experimental evidence is best described by a larger number of hydrogen bonds between water molecules in the interface as compared with the number of hydrogen bonds per moles of water in pure water (Némethy and Scheraga [7], *cf.* Némethy [8]).

From solubility data of amino acids (Nozaki and Tanford [9]) and hydrocarbons (Wetlaufer *et al.* [10] and the increase of critical micelle concentrations in detergent solutions (Bruning and Holtzer [11], Mukerjee and Ray [12]) it has been shown, that urea and similar compounds reduce hydrophobic bonding. This may be explained by a decrease of hydrogen bonding between water molecules in aqueous urea solutions as compared with hydrogen bonding in pure water (H. S. Frank and F. Franks, *cf.* Némethy [8]).

The reaction pathway of the tryptophan-hydrocarbon-photooxidation might depend on the solvation state of the hydrocarbon, the environmental water structure or both. Evidence about the influence of the environmental solvent structure alone is provided by studying the photoreaction in soap solutions containing urea in high concentration (6 M). In aqueous solutions of sodium dodecylsulfate containing 6 M urea 3,4-benzopyrene is still dissolved by the hydrophobic areas of the micelles, which are weakened but not completely broken up by addition of urea.

To study the influence of the solvation state of the hydrocarbon on the photoreaction, the soap is replaced by caffeine. Solubilization of aromatic hydrocarbons in aqueous solutions of purine derivatives like caffeine was first reported by Brock *et al.* [13] and studied in more detail by Weil-Malherbe [14], Liquori *et al.* [15], and Boyland and Green [16]. From absorption and fluorescence spectral shifts (Booth *et al.* [17], Boyland and Green [16], Reske and Stauff [18], Van Duuren [19]) it has been concluded, that caffeine and other purine derivatives dissolve polycyclic aromatic hydrocarbons by a dipole – induced dipole association mechanism between polar and polarizable molecules.

By exchange of soap against caffeine the solvation state of the hydrocarbon and the environmental solvent qualities are changed simultaneously, as the water structure in the vicinity of caffeine complexes is different from the water structure in the vicinity of soap micelles. To realize possible additional environmental changes the photoreaction is also studied in caffeine solution containing urea in the same concentration, as has been applied to change the qualities of the soap solution.

From the nature of the environmental change, that produces the shift to the sensitized tryptophan photooxidation, considerations concerning the solvation state of oxygen are derived, concerning the problem, how photooxidations and photosensitized oxidations, especially with respect to photodynamic action (for the literature see [20]), are influenced by the hydration of oxygen or excited oxygen. The results are further discussed in correlation with photoreactions of 3,4-benzopyrene and SH-compounds with different hydrophobic qualities [2], which might be of biological interest [21, 22].

Materials and Methods

DL-tryptophan, urea, and caffeine were purchased from Merck, Darmstadt (West Germany), sodium dodecylsulfate from Serva, Heidelberg (West Germany), and 3,4-benzopyrene from Fluka, Buchs (Switzerland), 3,4-benzopyrene laboratory purification (W. Hammer) was carried out by zone refining (80 zones), all other compounds were used without further purification. The solvent water was twice distilled.

The solutions were prepared similar to the procedure which was applied previously (Reske and Stauff [21]). Aqueous suspensions of 3,4-benzopyrene were given together with aqueous solutions of the soluble components. Tryptophan was added in solid form. After shaking overnight the solid excess was removed by repeated centrifugation and decantation. The centrifugations at 15,000 cycles per min were carried out with a temperature-adjustable Phywe-centrifuge using stainless steel sample-containers. To prepare the suspension of 3,4-benzopyrene in water a solution of the hydrocarbon in acetone was mixed with water. The organic solvent was removed by evaporation which was repeated several times after refilling with water. Before use the stock suspension was treated with ultrasonics for 1 min.

Absorption spectra were taken with a Beckman-DK 2a- and a Cary 15-spectrophotometer. Fluorescence spectra were obtained from a Zeiss spectrofluorometer ZFM 4 C with two prism monochromators and a 450 W xenon arc for exitation. A 6256 S EMI photomultiplier combined with a Zeiss PMQII-Metrawatt (Servogor) equipment was used for automatic recording. All spectra were taken at 25 °C using temperature adjustable cuvette holders.

The solutions were irradiated with a high pressure mercury lamp (Osram HBO 100 W/2) using a commercial lamp adjusting and focusing set (Spindler & Hoyer, Göttingen). An UG 2 filter (Schott & Gen., Mainz) of 5 mm pathway, which was water cooled, was used to remove visible light and the mercury-emission in the UV-range, were tryptophan absorbs. The light was focused into the cuvette, where the sample was stirred mechanically during irradiation. Behind the cuvette (1 · 1 · 4 cm, Suprasil, Hellma) an UG 11-filter (Schott) was placed to prevent visible light from benzopyrene-fluorescence from entering the photodiode, which was used together with a current amplifier (Dr. Steiger, Frankfurt/M.) for light intensity determinations. The photodiode was of a PNI-type, sensitive between 300 and 1200 nm, with a size of 1 cm^2, covering the whole area, which was illuminated in the cuvette. The cuvette holder was temperature adjustable. All

measurements were carried out at 25 °C. A solution of potassium ferrioxalate as actinometer (Parker [23], Hatchard and Parker [24], *cf.* Parker [25]) served for calibration.

For the determination of concentrations from the absorption of 3,4-benzopyrene its molecular extinction coefficients in sodium dodecylsulfate and caffeine solution had to be known. They were determined from solutions, which were prepared by diluting 1 cm^3 of a stock solution of $4 \cdot 10^{-4}$ M 3,4-benzopyrene in ethanol (Uvasol, Merck) to 100 cm^3 with ethanol, aqueous solution of 1% caffeine, or aqueous solution of 1% sodium dodecylsulfate respectively. The molecular extinction coefficients which were obtained from these solutions for corresponding absorption maxima were for ethanol $\varepsilon_{384.5\,nm} = 30000$, for dodecylsulfate solution $\varepsilon_{386\,nm} = 28800$ and for caffeine solution $\varepsilon_{390\,nm} = 22500$.

Results and Discussion

The change in the absorption spectra of aqueous solutions containing 3,4-benzopyrene and tryptophan on irradiation with light of the wavelength 366 nm is shown in Figs. 1 and 2. In Fig. 1 the influence of addition of 6 M urea on the reaction in aqueous solutions of sodium dodecylsulfate is demonstrated by comparison of Figs. 1 b and 1 c. In Fig. 1 a the reaction of 3,4-benzopyrene is shown, when no tryptophan or urea is added. Fig. 2 presents the corresponding series in aqueous solution of caffeine. The solutions were saturated in 3,4-benzopyrene and tryptophan respectively. The concentrations of tryptophan in its saturated solutions, which were determined by absorption spectrometry, were $1.5 \pm 0.3 \cdot 10^{-2}$ M, the hydrocarbon concentrations were between 0.7 and $1.5 \cdot 10^{-5}$ M.

The decrease of 3,4-benzopyrene during irradiation is seen in the decrease of the absorption bands of the hydrocarbon. As is shown in Figs. 1 a and 1 b the decrease of 3,4-benzopyrene becomes larger in solutions containing tryptophan. When 6 M urea is present (Fig. 1 c) this decrease is reversed, while the large increase of the tryptophan-photoproduct absorption remains unchanged. To estimate the formation of photoproducts the increase of absorption at 335 nm was evaluated. In caffeine solutions (Figs. 2 a – c) no larger decrease of benzopyrene but only increase of photoproducts is observed, when tryptophan is present, and no significant difference is observed, when the solution contains additional 6 M urea. Evidently the photocooxidation of benzopyrene and tryptophan in solutions of dodecylsulfate is replaced by a benzopyrene-photosensitized oxidation of tryptophan on addition of 6 M urea or in caffeine solutions.

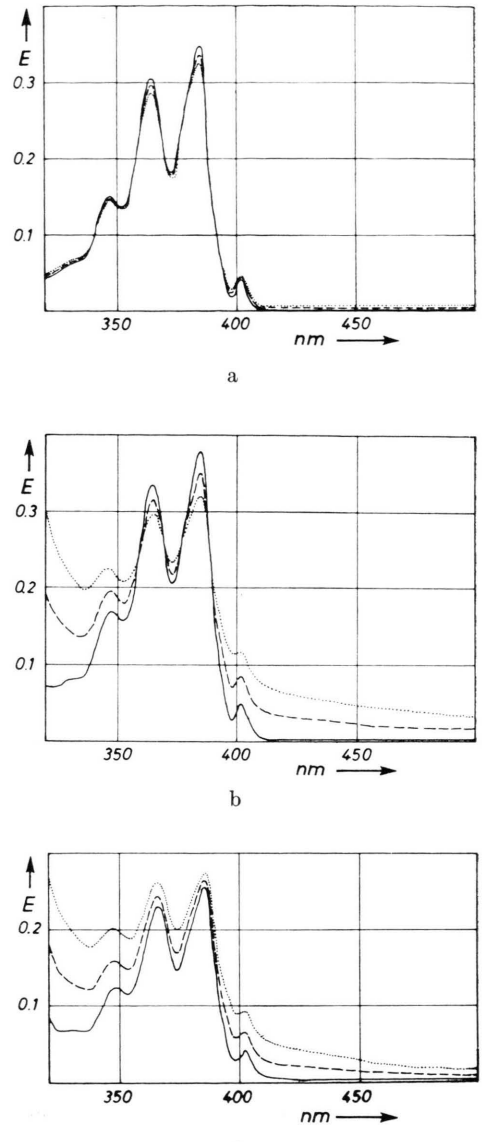
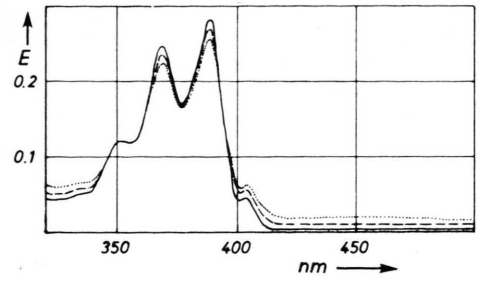
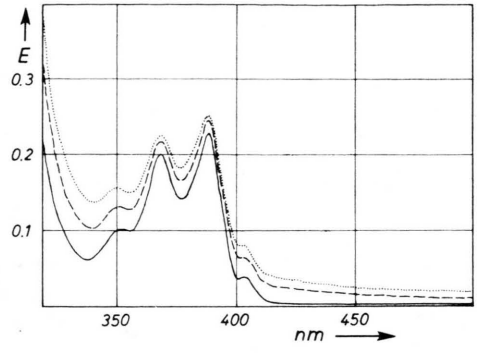


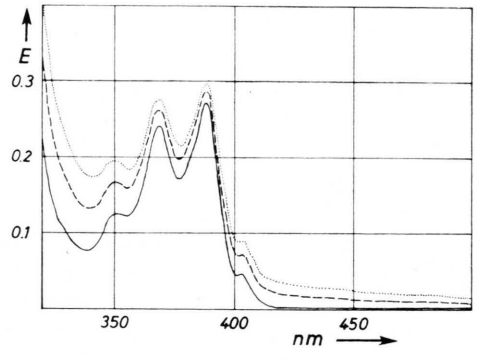
Fig. 1. Effect of irradiation (366 nm) on the absorption spectra of saturated solutions (25 °C) of 3,4-benzopyrene in aqueous 0.45% sodium dodecylsulfate; —— before, – – – after 20, ···· after 40 min of irradiation. a. No additive, reference: Aqueous 0.45% sodium dodecylsulfate; b. Saturated with DL-tryptophan, reference: Aqueous 0.45% sodium dodecylsulfate; c. Saturated with DL-tryptophan + 6 M urea, reference: Aqueous 0.45% sodium dodecylsulfate + 6 M urea.



a



b



c

Figs 2 a—2 c. As Figs 1 a, 1 b, and 1 c respectively with 0.5% caffeine instead of 0.45% sodium dodecylsulfate.

As long as the relation

$$-dc/dt = \Phi \cdot I_a = \Phi \cdot I_0 \cdot (1 - e^{-\varepsilon c})$$
$$\approx 2{,}3 \cdot \Phi \cdot I_0 \cdot \varepsilon \cdot c = \text{const.}\, c\,.$$

(c = concentration of 3,4-benzopyrene, Φ = quantum yield of 3,4-benzopyrene-decomposition, I_a = intensity of absorbed, I_0 = intensity of incident light, ε = molecular extinction coefficient of 3,4-benzopyrene)

holds, the oxidation of benzopyrene is of pseudo first order, because the concentrations of oxygen ($3 \cdot 10^{-4}$ M) and tryptophan are large against the concentration of the hydrocarbon and therefore remain constant during the reaction in the limits of the experimental error. In addition to that, the oxygen concentration was maintained constant by stirring the solutions in the open cuvettes during the irradiations. The increases of photoproduct absorption are observed to be of zero order in the whole range for the photosensitized reactions (Figs. 1 c, 2 b and c), as the benzopyrene decrease being small, the benzopyrene concentration can be taken as constant during the observation time. They are nearly of the same magnitude for the photoproducts in the three solvents in Figs. 1 c, 2 b and c (see Table I) and for the photoproducts of the cooxidation in the beginning (first 20 min, see Fig. 1 b and Table I). Later in the cooxidation reaction the increase of the absorption of the photoproducts becomes larger although the benzopyrene concentration is decreasing with cooxidation. This indicates a possible photosensitizing effect of photoproducts containing benzopyrene photocooxidation products. The absorption of photoproducts at 366 nm and the benzopyrene absorption at 335 nm has been taken into account in the evaluations of absorbed intensities and photoproduct absorption at 335 nm respectively.

Table I. Increase of photoproduct absorption ($E_{335\,nm}$) per 10^{-6} einsteins absorbed: ($\Delta E_{335\,nm}/10^{-6}$ einsteins$_{Abs.}$). Error: $0.3 \cdot 10^{-3}$.

Solvent \ Reaction system	3,4-Benzopyrene/O_2		3,4-Benzopyrene/DL-tryptophan/O_2	
0.45% sodium dodecylsulfate/water	$0.3 \cdot 10^{-3}$	(1 a)	$5.2 \cdot 10^{-3}$	(1 b)
0.45% sodium dodecylsulfate/6 M urea/water	$0.5 \cdot 10^{-3}$		$5.9 \cdot 10^{-3}$	(1 c)
0.5% caffeine/water	$1.7 \cdot 10^{-3}$	(2 a)	$6.1 \cdot 10^{-3}$	(2 b)
0.5% caffeine/6 M urea/water	$1.3 \cdot 10^{-3}$		$5.9 \cdot 10^{-3}$	(2 c)

The designations in parentheses refer to the corresponding Figs 1 and 2.

In caffeine solutions containing tryptophan a steep rise of absorption is observed below 335 nm towards shorter wavelengths already before irradiation. This rise, which is not seen in the corresponding soap solution spectra, is due to a shift of the

tryptophan absorption to longer wavelengths by the 0.5% caffeine content. The shift by high concentrations of caffeine was realized using cuvettes with 0.01 cm pathlength. In these cuvettes with saturated tryptophan solutions in water containing 0.05% caffeine against 0.5% caffeine solution in water as reference, tryptophan maxima were determined at 279.5 nm and 288 nm, which were at 278.5 nm and 287 nm respectively, when determined with the same solutions at a dilution rate with water of 1 : 100 in cuvettes with pathlengths of 1 cm. A small shift is sufficient to cause the strong rise of the absorption in Figs. 2 b and 2 c because of the high tryptophan absorption in these saturated solutions at 1 cm pathlength. The rise is merely additive and has no influence on the increase of absorption during irradiation within the experimental error.

The effect of caffeine and urea on the mechanism of the photoreaction is well seen, when the quantum yields of benzopyrene decomposition are compared (Table II). In soap solution containing tryptophan the quantum yield of benzopyrene oxidation is about 3.5 times that of the solution without tryptophan (Table II, first line). In caffeine solution the quantum yield of benzopyrene decomposition is smaller, when tryptophan is present than without tryptophan (Table II, third line), the latter being about 2.5 times the value of the corresponding soap solution. Addition of urea (Table II, second line) shifts the quantum yields from the values, which are observed in the soap solutions (Table II, first line), nearly to the same values, which are determined for the corresponding caffeine solutions (Table II, third line), where the effect of urea is within the limits of the experimental error (Table II, third and fourth line). The increase of the benzopyrene-decomposition on addition of urea to the soap solution or its higher amount in caffeine solution indicates a benzopyrene-sensitized photooxidation of benzopyrene, which is inhibited by competition of the benzopyrene-sensitized tryptophan-photooxidation, when tryptophan is present, as then the quantum yields of the benzopyrene-oxidation are not only smaller than the value for the soap solution (Table II, first line, second row) but also smaller than the values for the corresponding solutions without tryptophan (Table II, first row, second, third, and fourth line).

Table II. Quantum yields of the 3,4-benzopyrene photoreaction: (Moles 3,4-benzopyrene decomposed/einsteins absorbed by the hydrocarbon). Error: $\pm 0.05 \cdot 10^{-3}$.

Solvent \ Reaction system	3,4-Benzopyrene/O_2	3,4-Benzopyrene/DL-tryptophan/O_2
0.45% sodium dodecylsulfate/water	$0.22 \cdot 10^{-3}$ (1 a)	$0.76 \cdot 10^{-3}$ (1 b)
0.45% sodium dodecylsulfate/6 M urea/water	$0.49 \cdot 10^{-3}$	$0.34 \cdot 10^{-3}$ (1 c)
0.5% caffeine/water	$0.53 \cdot 10^{-3}$ (2 a)	$0.36 \cdot 10^{-3}$ (2 b)
0.5% caffeine/6 M urea/water	$0.59 \cdot 10^{-3}$	$0.36 \cdot 10^{-3}$ (2 c)

The designations in parentheses refer to the corresponding Figs 1 and 2.

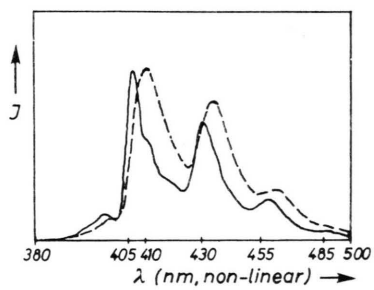


Fig. 3. Molecular fluorescence spectra of 3,4-benzopyrene (at concentrations below $2 \cdot 10^{-6}$, where for the geometry, which was applied, the reabsorption is smaller than the accuracy) in aqueous solution of sodium dodecylsulfate: full line; in aqueous solution of caffeine: broken line. In organic solvents the 3,4-benzopyrene fluorescence is strongly quenched by oxygen, for instance 60% in ethanol under atmospheric conditions. No oxygen quenching of the 3,4-benzopyrene fluorescence is observed in aqueous solutions (Weil-Malherbe [26], Stauff and Reske [27], Boyland and Green [16]), no matter whether the solubilization is brought about by purine derivatives, proteins, or soap. Under the assumptions, that the Stokes'-radii of oxygen and the lifetimes of fluorescence of 3,4-benzopyrene without quenching are the same in water and ethanol, using the Stern-Volmer-equation the steady state diffusion controlled dynamic oxygen quenching of the 3,4-benzopyrene fluorescence in water can be calculated from the solubilities and the diffusion coefficients of oxygen in water and ethanol, resulting in 18% quenching under air of 1 atm. The difference between this value and the observed 0% quenching is larger than the experimental error.

The absorption spectra in Figs. 1 and 2 and the fluorescence spectra in Fig. 3 show, that the main absorption and fluorescence band maxima of 3,4-benzopyrene are shifted about several nanometers to longer wavelengths in solutions of caffeine when compared with solutions in dodecylsulfate (Tables III and IV). When 6 M urea is added to the dodecylsulfate solution, a small shift of the benzopyrene band maxima is observed, which is scarcely above

Table III. Solvent shifts of 3,4-benzopyrene absorption band maxima. Error: ±0.5 nm.

Solvent	Wavelengths [nm]			
0.45% sodium dodecylsulfate/ water	403	386	365.5	348
0.45% sodium dodecylsulfate/ 6 M urea/water	404	387	366.5	349
0.5% caffeine/ water	405	390	370	351.5
0.5% caffeine/ 6 M urea/water	405	390	370	351.5

Table IV. Solvent shifts of 3,4-benzopyrene fluorescence band maxima. Error: ±0.5 nm.

Solvent	Wavelengths [nm]		
0.45% sodium dodecylsulfate/ water	406.5	430	456.5
0.45% sodium dodecylsulfate/ 6 M urea/water	407	431	457.5
0.5% caffeine/ water	411	434.5	460
0.5% caffeine/ 6 M urea/water	411	434.5	460

the experimental error (Tables III and IV). By addition of urea to the caffeine solutions the benzopyrene spectra are not changed at all. Accordingly as far as indicated by band shifts of the hydrocarbon, no change of the benzopyrene solution state is produced by urea, which could be compared to the effect of the soap against caffeine. Consequently, as far as this correlation is valid, the influence on the mechanism of the reaction is not due to changes in the solvation state of the benzopyrene molecule but to changes in the neighbouring water structure. As the changes in the quantum yields do not only occur in solutions containing tryptophan, but also when 3,4-benzopyrene is photooxidized alone, the only possibility left is a change in the solvation state of oxygen. This is taken into account by the following reaction scheme, which is consistent with the experimental evidence:

Photocooxidation:

$Bp \rightarrow Bp^*$

$Bp^* + O_2 \rightarrow (Bp \cdot O_2)^*$

$\downarrow + Tr$

Bp-, Tr-, Bp-Tr-photooxidation products

Bp = 3,4-benzopyerene
Tr = tryptophan

Photosensitized Oxidation:

$Bp \rightarrow Bp^*$

$Bp^* + O_2 + H_2O \rightarrow Bp + O_2^* \cdot H_2O$

$O_2^* \cdot H_2O + Tr \rightarrow$ Tr-photooxidation products

$O_2^* \cdot H_2O + Bp \rightarrow$ Bp-photooxidation products

Probably the triplet state of the hydrocarbon is involved in the photooxidations. For the cooxidation an intermediate of oxygen with the hydrocarbon is suggested. About the properties of the intermediate no statement can be made. The photosensitized oxidation is suggested to be carried on by excited oxygen, presumably $^1\Delta$-oxygen, in a hydrated state. It is conceivable, that hydrated oxygen formation is energetically more favourable, when the water structure is modified by caffeine or urea in a way, that hydrogen bond formation between water molecules is reduced, as is indicated by experimental evidence for aqueous urea solutions (see Introduction). Participation of $^1\Delta$-oxygen is suggested referring to the work of Merkel, Nilsson, and Kearns [28] and Nilsson, Merkel, and Kearns [29], who recently reported evidence on the role of $^1\Delta$-oxygen in the methylene blue sensitized photooxidation of tryptophan.

The influence of hydrophobic interaction and oxygen hydration on the benzopyrene-tryptophan photooxidation is further studied in experiments on the effect of electrolyte concentration, of deuterated water as solvent, on solvent dependence of benzopyrene triplet decay, and reactions with compounds, which inhibit the tryptophan photooxidation. With compounds containing SH-groups as the hydrophilic cysteine and the hydrophobic diphenylmercaptomethane different inhibition reactions have been observed, which correspond to the cooxidation (in the scheme above given) for the hydrophobic and to the photosensitized oxidation for the hydrophilic mercapto inhibitors (Reske [2, 22]).

The influence of hydrophobic interaction might be important in the carcinogenic and photodynamic activity of 3,4-benzopyrene and other polycyclic aromatic hydrocarbons [22]. Similar environmental changes of reaction pathways might also not only be valid, when the energy of the reaction is supplied by a process another than light excitation but also in carcinogenic reactions not involving hydrocarbons, as for instance with ionizing radiation. Correspondingly similar differences in reaction mechanisms might exist for the inhibition of radiation damage in correlation with its different consequences as carcinogenesis, mutation or cellular death.

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