DARK AND PHOTOINDUCED INTERACTIONS BETWEEN RIBOFLAVIN AND INDOLE AUXINS

Sandra MISKOSKI and Norman A. GARCÍA*

Departamento de Química y Física, Universidad Nacional de Río Cuarto, 5800 Río Cuarto, Argentina

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Interactions between riboflavin (RF) and indole auxins in the darkness and under conditions of irradiation with visible light were carried out. Dark complexation takes place in buffered (pH 7) aqueous solution. The association constants determined by static fluorescence quenching range from 70 to 150 mol^{-1} dm^3. The complexation is significant at relatively high indole auxin concentrations (above 10^{-3} mol dm^{-3}). It is governed by an additive effect of charge transfer, known for flavine–indole systems, and of the hydrophobic bond formation. The latter appears in the case of auxins as the driving force of the interaction. No quenching of singlet-excited RF was detected up to indole auxin concentrations of $5 . 10^{-3}$ mol dm^{-3}. Under irradiation with visible light a complex mechanism of competitive reactions is operative: both RF and especially the auxins are decomposed by a combination of type I and II photooxidations at indole auxins concentration of 10^{-4} mol dm^{-3}. The rate constants of the order of 10^9 mol^{-1} dm^3 s^{-1} were estimated for the quenching of triplet excited RF by the indoles. For comparative purposes Rose Bengal sensitized photooxidation of a series of indolecarboxylic acids of indole-3-acetic, -propionic and -butyric acid was examined. The rate constants of the photooxidative process, mediated by singelt molecular oxygen, were of the order of 10^7 mol^{-1} dm^3 s^{-1} for the auxins. As a result of the interaction, RF is protected against photobleaching and the auxins decompose via a different mechanisms, depending on the relative flavin–auxin concentrations.

Flavins are probably of all biomolecules most extensively studied with respect to their ability of complexation with other compounds of biological relevance. Indole-3--alkanoic acids (I_3ca), possessing an aliphatic chain of 1–4 carbon atoms, belong to the family of plant growth hormones generally named auxins. The simultaneous presence of auxins and flavin derivatives in plants has aroused the interest of researchers especially as regards mutual interactions of these biomolecules and their photophysiological implications since flavins are potential plant photoreceptors of solar irradiation.

The great number of papers on dark complexation[1,2] and on interactions of photoexcited flavins[3–5] with several indole derivatives, including flavin-sensitized photooxidations[4–6] of different substrates, point to the biological importance of this topic. Charge transfer interactions of type I (radical-mediated) and type II (singlet molecular oxygen $[O_2(^1\Delta_g)]$-mediated) photooxidations have been postulated to

control the mechanisms of these reactions. We are presenting here an integral study on the complex interaction of RF and indole auxins in the dark and under conditions of irradiation with visible light, including a systematic examination of flavin and the indole derivatives substituted in different ways.

The present investigation was undertaken in an effect: to (i) evaluate the conditions under which the different reaction mechanisms operate and (ii) to characterize the influence of the molecular structure of the auxins on the interactions observed. The results indicate that an important dark association takes place between RF and indoles. Moreover, under irradiation, reactions of auxins with excited triplet RF and with photochemically generated O_2 ($^1\Delta_g$) takes place. The relative importance of these reactions was also evaluated. Rose bengal (RB) sensitized photooxidation of the indoles was employed for comparative purposes. The rate constants of the interaction of auxins with O_2 ($^1\Delta_g$) are reported for the first time.

EXPERIMENTAL

Indole (Ind), indole-2-carboxylic acid (I_2C), indole-3-carboxylic acid (I_3C), indole-5-carboxylic acid (I_5C), indole-3-acetic acid (I_3A), indole-3-propionic acid (I_3P) and indole-3-butyric acid (I_3B), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and riboflavin (RF) were purchased from Sigma. RF was purified according to Silber et al.[7]. Riboflavin tetrabutyric acid (FTB) was a gift of Prof. P. S. Song. Rose Bengal (RB) was from Aldrich and histidine from Fluka. The solvents were of HPLC quality. Water was three times distilled. Phosphate buffer at pH 7 was employed.

The irradiation device[8] as well as the oxygen electrode[9] were described previously. Experiments under anaerobic conditions were made in the presence of ultrapure Argon (bubbling for 30 min) in the cuvet of 1 cm path length). For the absorption measurements a Cary 17 or a Hewlett Packard 8452A Spectrophotometer were employed. Fluorescence measurements were carried out in an Aminco SPF 125 Spectrofluorometer.

Fluorescence lifetime measurements were performed with a nitrogen laser (Laser Optics S.A.) gated at 50 Hz (FWHM 3·5 ns) at 337 nm as excitation source; a Hewlett Packard digital oscilloscope interfaced to an IBM microcomputer was used to monitor the signal from a TRW 31A fluorometer[10]. RF fluorescence lifetimes (in the absence (τ_0) and in the presence (τ) of I_3ca) were calculated by the phase plane deconvolution method[11].

The rate constants for the sensitized photooxidation of indoles were determined by means of a modified version of the method introduced by Foote and Ching[12]. The ratio of the first order plots of oxygen uptake by the reference compound and by the indoles was evaluated for the calculation of the oxidation rate constants.

RESULTS

Dark Complexation

The addition of a series of different I_3ca's to aqueous solutions of RF causes similar changes in the absorption spectrum of the dye. There is a decrease in the absorption

of the main flavin band (450 nm) accompanied by a smooth increase of the absorption on the long wavelength side of that band. These spectral perturbations can be clearly observed in the difference spectra shown in Fig. 1 in the case of I_3B. The amplitude of the difference band at 450 nm increases with the length of the side chain of the 3-substituted indole compounds. A solvent effect on the magnitude of the spectral changes was observed: the lower polarity of the solvent the smaller the difference band.

The result presented above confirms the observed tendency of RF to form molecular complexes with several types of biological substrates including indoles such as 2-methylindole, 3-methylindole, tryptophan and I_3A (refs[1,2]). The association constants (K_{ass}) range from 15 to 40 mol^{-1} dm^3 with an unexpected value of 603 mol^{-1} dm^3 for 2-methylindole[1]. In the present study we employed the routine procedure of fluorescence quenching to obtain the apparent association constants. RF is characterized by strong fluorescence emission at 515 nm (uncorrected) which was quenched by the series of I_3ca's of varying efficiency. Assuming that the quenching does not proceed via interaction with the excited state of RF, the apparent association constants for complexing in the ground state can be calculated from the Stern–Volmer plots.

$$I_0/I = 1 + K_{sv}[Q] , \qquad (1)$$

where I_0 and I are values of fluorescence intensity in the absence and in the presence of the quencher (Q) respectively; K_{sv} denotes the association constant.

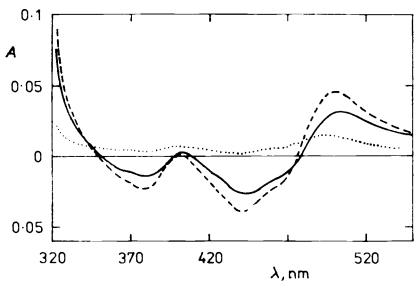

FIG. 1

Difference spectra of RF–I_3B in water, pH 7. RF, $A_{445} = 0{\cdot}7$ (.... 1 mM, — — — 5 mM, — 25 mM)

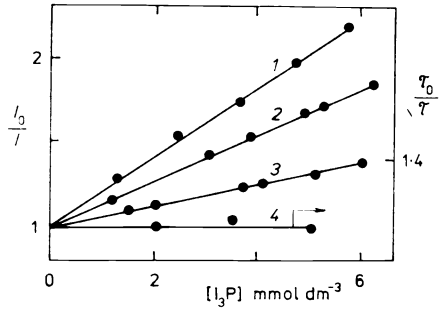

FIG. 2

Stern–Volmer plots for static (axis I_0/I) and dynamic (axis τ_0/τ) fluorescence quenching of flavins by I_3P. *1* RFTB in Bz; *2*, *4* RF in water, pH 7; *3* RF in EtOH–water, 1 : 1 by vol.

In order to elucidate the influence of solvent polarity on K_{sv} and structural effects on the fluorescent probe and quenchers, experiments with different flavins (RF, FMN, FAD, RFTB) and indoles with rings substituted in different positions were carried out in several solvents and solvents mixtures. In all cases linear Stern–Volmer plots were obtained. The K_{sv} values are listed in Table I. In parallel experiments the time-resolved fluorescence of RF in the absence and in the presence of indoles was examined. The effect on the time resolved fluorescence signal of RF could not be detected up to indole concentrations of $5 \cdot 10^{-3}$ mol dm^{-3}. A radiation halflife of 4·2 ± 0·1 ns was determined for RF in H_2O (pH 7) in agreement with recorded data[13,14]. Hence, the observed inhibition of RF fluorescence measured in static experiments can be totally ascribed to an association in the ground state. Consequently, I_3ca's must be complexed with RF prior to excitation. Typical plots of static fluorescence quenching of several flavins and time resolved results are shown in Fig. 2.

Light Promoted Interactions

The results will be presented and analyzed on the basis of this reaction scheme:

$$RF \xrightarrow{h\nu} {}^1RF^* \qquad (2)$$

$${}^1RF^* \xrightarrow{k_{d1}} RF + h\nu' \qquad (3)$$

$${}^1RF^* \xrightarrow{k_{ISC}} {}^3RF^* \qquad (4)$$

TABLE I

Stern–Volmer constants (K_{sv}, mol^{-1} dm^3, ±3%) for fluorescence quenching of RF, FMN, FAD and RTFB in several solvents by indole derivatives at 25°C

Indole derivative	RF[a]	FMN[a]	FAD[a]	RFTB[b]	RF[c]
Indole	80				44
I_2C	127				57
I_5C	118				52
I_3C	70	25	21		38
I_3A	93	39			46
I_3P	130			200	62
I_3B	150	51	45	198	72

[a] H_2O, pH 7; [b] benzene; [c] MeOH–H_2O, 1 : 1 by vol.

$$^3RF^* \xrightarrow{k_{d3}} RF \qquad (5)$$

$$^3RF^* \xrightarrow{k_{PI}} P_I \qquad (6)$$

$$^3RF^* + I_3ca \xrightarrow{k_{PII}} P_{II} \qquad (7)$$

$$^3RF^* + {}^3O_2 \xrightarrow{k_{ET}} RF + O_2(^1\Delta_g) \qquad (8)$$

$$O_2(^1\Delta_g) + RF \xrightarrow{k_{ox}} P_{III} \qquad (9)$$

$$O_2(^1\Delta_g) \xrightarrow{k_d} {}^3O_2 \qquad (10)$$

$$O_2(^1\Delta_g) + I_3ca \xrightarrow{k_q} {}^3O_2 + I_3ca \qquad (11)$$

$$O_2(^1\Delta_g) + I_3ca \xrightarrow{k_r} P_{IV} \qquad (12)$$

where the following steps are: (*2*) absorption of radiation by RF; (*3*) fluorescence; (*4*) intersystem crossing; (*5*) thermal deactivation from the excited triplet state; (*6*) bleaching reaction of RF; (*7*) quenching of the excited triplet state of RF by I_3ca; (*8*) energy transfer to ground state oxygen ($^3O_2$) and generation of singlet molecular oxygen; (*9*) photooxidation of RF; (*10*) thermal deactivation of $O_2(^1\Delta_g)$; (*11*) physical quenching of $O_2(^1\Delta_g)$ and (*12*) reactive quenching (photooxidation) of I_3a. P_I to P_{IV} represent different photoproducts. The sum $k_q + k_r$ will be denoted by k_t, the overall rate constant accounting for the total quenching process $O_2(^1\Delta_g)$.

The irradiation of RF under both aerobic and anaerobic conditions leads to a different series of chemical reactions. The ribityl side chain is cleaved, the isoalloxazine ring is also affected and bleaching of flavin takes place[15,16]. It is known that RF photodegradation proceeds in alcohols from the singlet excited state while the triplet pathway[13] is favoured in water.

The rates of photolysis can be easily determined spectroscopically in terms of decrease in RF band at 445 nm. In the presence of the I_3ca's in concentrations lower than $1 \cdot 10^{-3}$ mol dm^{-3} (sufficiently low to avoid dark complexation) the rate of RF uptake was drastically diminished in water at pH 7. Simultaneously an increase in the 375 nm band appeared as a result of the absorption by reaction products, as shown in Fig. 3 for I_3P. Qualitatively, both the RF absorptions (sensitizer) and the I_3ca, absorptions decreased after irradiation of the mixture at wavelengths higher than 400 nm. These results strongly support the idea[16] that a long-lived triplet state, which is quenched by very low concentrations of I_3ca, is the intermediate in RF photolysis.

The rates of I_3A, I_3P and I_3B photodestruction through RF sensitized photolysis were determined as the initial slopes of the curves presented in Fig. 4 (inset). The loss of the indole derivative as a function of irradiation time was monitored using the Salkowsky reagent[6].

Under argon the rate of RF photobleaching was much faster than under saturation with air. Simultaneously the rate of anaerobic RF decomposition was substantially decreased in the presence of I_3ca. Assuming that reaction (7) is the unique source of this quenching, we make a rough calculation of k_{PII}. The results can be evaluated using the simple Stern–Volmer equation (13).

$$V_0/V = 1 + k_{PII}\tau_3[I_3ca] , \qquad (13)$$

where V_0 and V represent the velocities of anaerobic RF uptake in the absence and

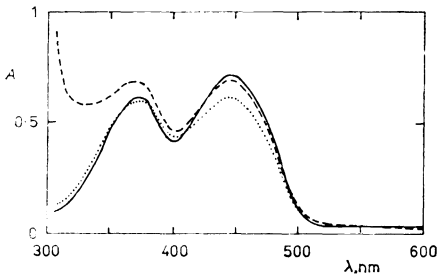

FIG. 3

Absorption spectra: unphotolyzed RF (—); RF after 10 min of photolysis (....); RF + I_3P 5 . 10^{-4} mol dm^{-3} after 10 min of photolysis (— — —). The spectrum of unphotolyzed (– – – –) is equal to (—) in the region 400—600 nm

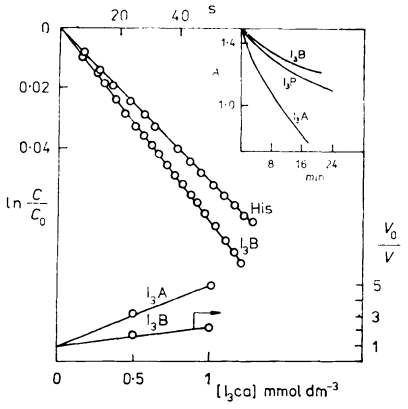

FIG. 4

Stern–Volmer plots for inhibition of deoxygenated RF photobleaching in water at pH 7 by auxins and first order plots for oxygen uptake upon sensitized irradiation of air-saturated aqueous solutions (pH 7) containing alternatively: Histidine and I_3B 1 . 10^{-4} mol dm^{-3} plus sensitizer (RB, $A_{560} = 0{\cdot}5$). C and C_0 are the respective concentrations of $^3O_2$ of the photolyzed and unphotolyzed solutions. Inset: Absorbance vs irradiation time for photodecomposition of I_3ca, determined according to ref.[6]. Initial absorbance values were made equal to 1·5 .

in the presence of I_3ca as measured by the decrease in the absorbance of the 445 nm band. Taking the value of 14 μs as the triplet lifetime of RF[17] (τ_3) we determined graphically the k_{PII} values as $3 \cdot 10^9$, $1{\cdot}5 \cdot 10^9$ and $1 \cdot 10^9$ mol^{-1} dm^3 s^{-1}, for I_3A, I_3P and I_3B, respectively. The corresponding plots for I_3A and I_3B and two auxins concentrations are shown in Fig. 4. The k_3 values are of the same order of rate constant magnitude (3·9 mol^{-1} dm^3 s^{-1}) as that reported by Yoshimura and Ohno[5] for the interaction of triplet lumiflavin with indole in MeOH.

RF is a relatively efficient generator[18] of O_2 ($^1\Delta_g$) provided that adequate irradiation under aerobic conditions is applied. Chacon et al.[18] determined a quantum yield of 0·48 in MeOH for process (*8*), the rate constant (k_{ox}) for the O_2-quenching ($^1\Delta g$) by the proper dye being $6 \cdot 10^7$ mol^{-1} dm^3 s^{-1}. On the other hand indole derivatives, and I_3ca in particular, are well known O_2 ($^1\Delta_g$) quenchers[4-6,19-23].

In order to evaluate the potential contribution of singlet molecular oxygen-mediated photooxidation of the RF-sensitized auxins, relative measurements of oxygen uptake were performed. Rose Bengal (with a quantum yield equal 0·81 for O_2 ($^1\Delta_g$) generation in water[24]) was employed as sensitizer of a series of indoles, including I_3ca's. Histidine was used as a reference.

$$O_2\,(^1\Delta g) + \text{His} \xrightarrow{k_{rH}} P_V \qquad (14)$$

It is known[25] that process (*14*) is a totally reactive interaction with a reported k_{rH} value of $8{\cdot}8 \cdot 10^7$ mol^{-1} dm^3 s^{-1}.

From the ratios of the first order slopes for His and the substrates, the k_r values for different I_3ca were obtained (Fig. 4 and Table II). The only piece of information comparable with, our data is the k_t value reported by Mishoshi et al.[4] for I_3A i.e.

TABLE II

Rate constants for chemical reaction (k_r, mol^{-1} dm^3 s^{-1}) of O_2 ($^1\Delta g$) with indole derivatives (sensitizer: RB, $A_{560} = 0{\cdot}6$) and relative rates (V_{ox}) for oxygen uptake (sensitizer: RF, $A_{445} = 0{\cdot}7$)

Indole derivative	$k_r \cdot 10^{-7}$	V_{ox}
RF		0·01
Indole		0·7
I_3C	1·4	0·21
I_3A	9·2	1·0
I_3P	7·3	0·7
I_3B	7·2	0·7

$1 . 10^9$ mol^{-1} dm^3 s^{-1} at pH 7. The k_q/k_r ratio for the same indole was recentyl reported[20] by our group. The calculated k_r value from ref.[4] is $8 . 10^7$ mol^{-1} dm^3 s^{-1}, in good agreement with our data (Table II).

Supporting that approximately 20% of $O_2^{\cdot-}$ is produced by quenching of RB in triplet state by triplet ground state oxygen in water[26], the reactive rate constants implicity receive a contribution from this intermediate. As a consequence, the k_r values in Table II should be considered upper limits.

In parallel experiments the O_2 uptake with RF as a sensitizer was studied. As shown by the relative rates given in Type II, RF (initial concentration $5 . 10^{-5}$ mol . . dm^{-3}) itself is characterized by a low rate of O_2 uptake upon irradiation which is almost negligible in the presence of indoles (initial concentration of $5 . 10^{-4}$ mol . . dm^{-3}). Both reactions responsible for oxygen uptake (RF alone and RF plus indoles) were completely suppressed in the presence of 10^{-3} mol dm^{-3} concentration of NaN_3.

DISCUSSION

Dark Complexation

The association between tryptophan, some substituted indoles and RF has been ascribed by several authors[3,5], exclusively to a charge transfer (CT) interaction with RF acting as electron acceptor. These complexes however, do not satisfy the operational definition of a CT interaction, i.e. the appearance of a new absorption band in the spectra of the mixture indole derivative–RF. In these cases, similarly as in present experiments, the visible spectra of the complexes showed RF absorption tailing into the long-wavelength region.

The formation of a dark complex between RF and I_3A was reported by Nathanson et al.[6], but no data on the magnitude of the association constant and no discussion of the mechanism of the interaction were presented. In earlier studies Wilson[3] investigated the association of FMN and FAD with some indole derivatives including I_3A. A value of 40 mol^{-1} dm^3 was reported for the association constant determined by absorption spectrometry; this value is in excellent agreement with our data (39 mol^{-1} dm^3 $\pm$ 3%, Table I) obtained by fluorescence quenching. The interaction was explained by charge transfer forces[6].

Although some controversy exists regarding the relation between the spectral perturbations observed and the formation and stabilization of a given CT complex, we assume, in agreement with former proposals[1-3,6], that the dark RF–indole interaction is at least partially governed by a CT complexation mechanism. Indoles have an extended π-electron system, and consequently are able to form some sort of π-molecular complexes of reasonable stability with ground state electron acceptors. The relative magnitude of K_{sv} for indole (Table I) indicates that the interaction of the

aromatic indole ring with RF is responsible for the primary driving force of the association. The occurence of a CT interaction is supported by the following pieces of evidences: *a*) the existence of defined difference spectra (Fig. 1), dependent on the concentration of both RF and I_3ca; *b*) the excellent linearity of the Stern–Volmer plots for quenching of RF fluorescence, indicating unique type of interaction[27]; *c*) the absence of quenching of $^1RF^*$ by I_3ca's up to concentrations of $5 . 10^{-3}$ mol . $. dm^{-3}$; *d*) the correlation between the increase in K_{sv} for a given donor (Table I) in the series FAD–FMN–RF, and the order of decrease of the reduction potential for these flavins[28-30] at pH 7.

It has been generally accepted that CT does not make an appreciable contribution to the stability of the CT complex itself. Furthermore, the existence of CT forces does not exclude the possibility that other type of interactions may also be involved in the formation of these complexes. This assumption and the increase in K_{sv} with the increasing length of the hydrocarbon side chain observed with I_3ca's, seem to suggest the possibility of an hydrophobic interaction playing a partial role in the RF I_3ca association. In our present experiments this interaction can be of primary importance for the driving force of molecular association in aqueous solutions. The decrease in K_{sv} values with decreasing water content in the mixture and the presence of the effect due to the $-CH_2$ groups in the solvent mixture seem to support the above postulation.

If the same type of interaction as with the remaining flavins is assumed, the increase in K_{sv} for RF in a nonpolar solvent such as Bz can be ascribed to a favored orientation or to RFTB–I_3ca overlapping the CT. As can be expected, no hydrophobic contribution to K_{sv} was observed in such cases (Table I). In variety of theoretical reactivity indexes including total π-electron densities and localization energies, indicate that position 3 of the indole ring is the preferred site for electrophilic substitution, thus possessing a higher electron density[31]. It is not surprising that position 3 is considerably involved in the formation of the proposed π-complexes with flavins as electron acceptors. Since the substitution on C_3 by the electron releasing carboxylic group diminishes the electron density in this position, we can predict differences in K_{sv} found for the series $I_2C \approx I_5C > I_3C$, where the hydrophobic contribution should be the same.

Photochemical Interaction

The results concerning RF and decomposition of auxins will be discussed separately:

A) RF Decomposition

In aqueous solutions saturated with air, RF decomposes by a combination of two competitive mechanisms: bleaching as a result of transition from the excited triplet state (step (*6*)), with concomitant production of lumichrome[15,16], and photooxidation by O_2 ($^1\Delta_g$), produced by RF itself (steps (*8*) and (*9*)).

In the presence of I_3ca two quenching processes take place, involving as mentioned earlier the excited state triplet RF. Anaerobic quenching leads to a high efficiency as evidenced by the values of rate constants (k_{PII}) which are of the order of 10^9 mol^{-1} . . dm^3 s^{-1} (see Results), as determined by static photolysis. The process is most likely similar to that reported for the system triplet excited lumiflavin–indole, with intermediates well characterized by flash photolysis[5].

Under aerobic conditions, besides reaction (*6*), RF undergoes reaction also with O_2 ($^1\Delta_g$) (process (*9*)). This was demonstrated by the strong inhibition of oxygen uptake during the RF-sensitized photooxidation of auxins in the presence of NaN_3 (a recognized singlet molecular oxygen quencher[8]).

In the presence of I_3ca's competitive reactions (*7*) and (*8*) take place. Considering a mean value (see Results) of $1{\cdot}6 . 10^9$ mol^{-1} dm^3 s^{-1} for k_{PII}, $7{\cdot}1 . 10^{-1}$ for k_{ET}, which is 1/9 of the diffusion-controlled rate constant in water[32,33] and a value of $2{\cdot}65 . 10^{-4}$ mol dm^{-3} for oxygen concentration[34] in water saturated with air, reaction (*8*) predominates only at concentrations of I_3ca's much lower than 10^4 mol . . dm^{-3}.

Under conditions leading to O_2 ($^1\Delta_g$) formation reactions (*9*) and (*11*) + (*12*)) become competitive. In such cases, as evidenced by their rate constants (see Results) (mean value[4] of $1 . 10^9$ mol^{-1} dm^3 s^{-1} for k_t of I_3ca and typical concentration of $5 . 10^{-5}$ mol dm^{-3} for RF), O_2 ($^1\Delta_g$) photooxidation of the dye proceeds only in condition that the I_3ca concentration is of the order of 10^{-5} mol dm^{-3}.

B) I_3ca Photodestruction

Nathanson et al.[6] studied the mechanism of I_3A photodestruction, sensitized by RF in aerated aqueous solutions. The authors reported a quantum yield of 0·71 for this process using concentrations of $1 . 10^{-4}$ and $2 . 10^{-5}$ mol dm^{-3} for I_3A and RF, respectively. The mechanism is described as a unique reaction between $^3RF^*$ and I_3A with indole aldehyde as the only reaction product. It should be pointed out that I_3A conversions of the order of 30% were assumed in these calculations. Under the conditions mentioned, the authors are possibly describing the global process of I_3A consumption but certainly not a detailed mechanism of auxin photooxidation. According to our kinetic data presented earlier, the experimental conditions employed by Nathanson et al.[6] are precisely those of reactions (*7*), (*8*) and (*10*)–(*12*). The dark complexation must be disregarded because of the low concentration of both I_3ca's and RF. The same holds for reaction (*9*). Mishoshi et al.[4] came to similar conclusions on the FMN-sensitized photooxidation of I_3A. Under aerobic conditions the reactions of auxin with $^3FMN^*$ and photooxidation via O_2 ($^1\Delta_g$) are the main processes. In a concentration range similar to that used in the present study, both type I and type II reactions take place. The ratio of their relative velocities (V) was highly dependent on pH and reached a plateau between 6 and 8, the value of V_I/V_{II}

being approximately 0·2. No data on the rate constant of type I reaction were reported.

The plots in Fig. 4 (inset) characterising photodestruction of I_3ca indicate that the order of increase of the rate of RF-sensitized photodecomposition is qualitatively similar to the order of the respective rate constant for $^3RF^*$ quenching. On the other hand, the rate of oxygen uptake by RF-sensitized I_3ca's is similar (Table II) to the rate constant (k_r) for reaction with O_2 ($^1\Delta g$). We interpret these findings as follows: both interactions (reactions (*7*) and (*11*)) take place under our experimental conditions (similar to ref.[6]). Apparently, the driving interaction is reaction (*7*). The contribution of reaction (*11*) becomes operative only when oxygen uptake is being dosed up.

Our results are qualitatively in agreement with the findings published recently by Amat-Guerri et al.[35]. The authors reported on the distribution of products of RF- and RB-photooxidation of I_3A. Both direct and RF-sensitized irradiations yield essentially the same products yet somewhat different from those obtained by the RB sensitized process in which the O_2 ($^1\Delta g$) mechanism predominates.

As a final conclusion we can state that besides the dark association of RF with auxins at concentrations higher than $1 \cdot 10^{-3}$ mol dm^{-3}, a complex process takes place in the presence of visible light and at lower auxin concentrations. The main effect observed is the protection of RF from photobleaching by quenching of its triplet excited state by auxins. The photodestruction of the indole derivatives in parallel process is governed by type I and type II mechanism.

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