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Laser Flash Photolysis and Time-Resolved CIDNP Study of Photochemical Reactions between Aqueous Tryptophan and Nucleotides

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Abstract. Triplet tryptophan ^{T}TrpH quenching the by nucleotides adenosine-5′-monophosphate (AMP), cytidine-5′-monophosphate (CMP), guanosine-5′-monophosphate (GMP), and uridine-5′-monophosphate (UMP) is reported for the first time. The quenching rate constants for AMP, CMP, and UMP are of the same order $k_q = (3–6) \cdot 10^8$ $M^{-1}s^{-1}$, for GMP this value is $5.6 \cdot 10^7$ $M^{-1}s^{-1}$. This difference correlates with the reduction potentials of the nucleotides under study, pointing at the involvement of the electron transfer process. ^{T}TrpH quenching by AMP, CMP, and GMP does not result in the radical formation, whereas in the reaction of ^{T}TrpH with UMP radicals are formed with the quantum yield $\Phi \approx 0.13$, the radical formation is confirmed by both laser flash photolysis and time-resolved chemically induced dynamic nuclear polarization measurements.

1 Introduction

Photochemical reactions of amino acids and nucleic acid bases have been the subject of numerous studies [1–7]. In living cells, photooxidation of proteins can occur by three main mechanisms: (i) photoinduced generation of singlet oxygen and subsequent reactions of this species, (ii) light absorption by chromophores bound to protein, and (iii) light absorption by amino acid residues in proteins. The last mechanism corresponds mostly to the aromatic amino acids which absorb light in the near-UV range: tryptophan, tyrosine, phenylalanine, and, to a lesser extent, histidine. The properties and chemical reactions of photoexcited states of aromatic amino acids have been intensively studied over the last decades [1–3, 5, 8–10].

The photochemical damages induced in DNA are believed to be the most dangerous consequence of the UV irradiation for living matter. Fortunately, nucleic acid bases have a rather weak absorption in near UV, and photoinduced DNA lesions usually correspond either to irradiation by light with wavelengths

shorter than 260 nm, or to the sensitized photolysis. In particular, it has been recently shown that acetone-sensitized photolysis of nucleotides results in the formation of nucleotide triplet states and radicals arising from electron transfer reactions [11–14].

In this respect, the investigation of photochemical reactions between aromatic amino acids and nucleotides may present a special interest. Living cells contain high abundance of proteins, and in the cell nuclei, proteins are often bound to DNA molecules. Thus, short-lived intermediates formed in the photolysis of amino acids (triplet states, solvated electrons, radicals) may react with nucleobases and cause the DNA lesions.

Tryptophan is the strongest chromophore among the major amino acids present in proteins. Its absorption extends above 300 nm and reaches the range of solar UV light ($\lambda > 290$ nm). The photochemistry of tryptophan has been studied in detail [1, 3, 10, 15–24]. The primary processes in the direct tryptophan photolysis are the photoionization occurring from the nonrelaxed prefluorescent state [19, 20, 22, 24] and the relaxation into the excited singlet S_1 state. The main channels of S_1 decay are fluorescence, intersystem crossing into the triplet state T_1, and intramolecular proton transfer from the amino group to the excited indole ring [10, 17–19, 24–26]. Two of the intermediates formed in the tryptophan photolysis, solvated electron and triplet tryptophan, are sufficiently reactive and long-lived to be able to react with nucleic bases.

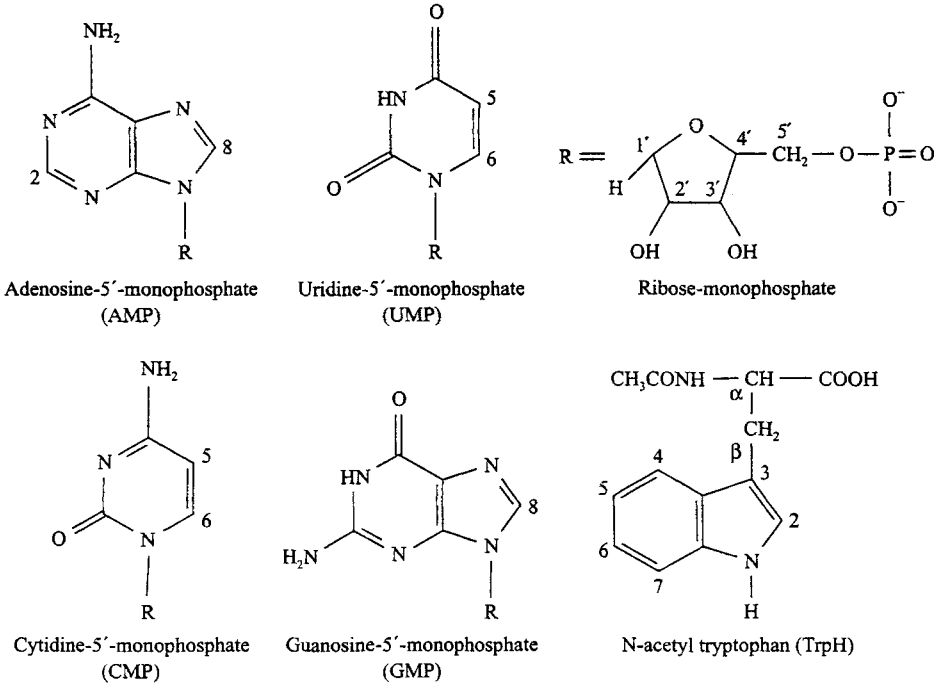

Fig. 1. Structures of N-acetyl tryptophan and nucleotides.

The reactions between solvated electrons and nucleotides were studied previously by means of pulse radiolysis [6, 27]. Both purine and pyrimidine nucleosides readily react with e^-_{aq} with diffusion-controlled second-order rate constants of about 10^{10} $M^{-1}s^{-1}$, although on introduction of the phosphate group the rate constants decrease due to the electrostatic interaction between e^-_{aq} and nucleotide [28]. In DNA the negatively charged center (anion radical) migrates to a final location at thymine or cytosine [29–32], the migration process is governed by the reduction potentials and pK_a values of the nucleic bases [33–35].

In the present paper, we report the first observations of the reactions between triplet tryptophan, formed in 308 nm laser photolysis of N-acetyl tryptophan (TrpH), and nucleotides adenosine-5′-monophosphate (AMP), cytidine-5′-monophosphate (CMP), guanosine-5′-monophosphate (GMP), and uridine-5′-monophosphate (UMP) (Fig. 1). The study is performed with the use of laser flash photolysis and time-resolved (TR) chemically induced dynamic nuclear polarization (CIDNP). The main goal of the study is to determine whether nucleotides can quench triplet tryptophan and to establish the quenching mechanisms.

2 Materials and Methods

Laser Flash Photolysis. A detailed description of the laser flash photolysis (LFP) equipment has been published earlier [36, 37]. Solutions in a rectangular cell (10 by 10 mm) were irradiated with a Lambda Physik EMG 101 excimer laser (308 nm, pulse energy of up to 100 mJ, pulse duration of 15–20 ns). The dimensions of the laser beam at the front of the cell were 3 by 8 mm. The monitoring system includes a DKSh-150 xenon short-arc lamp connected to a high-current pulser, a homemade monochromator, a 9794B photomultiplier (Electron Tubes Ltd.), and a LeCroy 9310A digitizer. The monitoring light, concentrated in a rectangle with a height of 3 mm and a width of 1 mm, passed through the cell along the front (laser-irradiated) window. Thus, in all experiments the excitation optical length was 1 mm, and the monitoring optical length was 8 mm. All solutions were bubbled with the appreciate gas (Ar, N_2O) for 15 min prior to, and during, irradiation. All experiments were carried out at room temperature in buffered solutions.

TR-CIDNP. A detailed description of the TR-CIDNP experiment has been reported elsewhere [38]. A sample purged with argon (or with N_2O when needed) and sealed in a standard nuclear magnetic resonance (NMR) pyrex ampoule was irradiated by a Compex Lambda Physik excimer laser (wavelength of 308 nm, pulse energy of up to 150 mJ) inside the probe of an Avance-200 Bruker NMR spectrometer. TR-CIDNP experiments were carried out by the usual pulse sequence: presaturation, laser pulse, evolution time-radio frequency pulse, free induction decay. As the background signals in the spectrum are suppressed by the presaturation pulses, only signals of the polarized products formed during the variable delay between the laser and NMR radio-frequency (rf) pulse appear in the CIDNP spectra. For kinetic measurements a 1 μs rf pulse was used. The

samples for CIDNP measurements were prepared by dissolving the nucleotide and tryptophan in D_2O. The pH values of deuterated solutions were adjusted by addition of DCl or NaOD and were measured by a glass electrode and not corrected for isotope effects.

Chemicals. N-acetyl tryptophan, guanosine-5′-monophosphate, cytidine-5′-monophosphate, uridine-5′-monophosphate, adenosine-5′-monophosphate, acrylamide, NaOD, DCl, D_2O were used as received from Sigma/Aldrich. KBr was purified by recrystallization. H_2O was doubly distilled. Buffers were prepared with sodium phosphate (pH 7.0).

3 Results and Discussion

3.1 LFP Measurements

The irradiation of aqueous solutions of TrpH at neutral pH results in the formation of intermediate reactive species with the following spectral features: solvated electron e_{aq}^- has a broad absorption spectrum with the maximum near 720 nm [39], triplet tryptophan ^{T}TrpH has a maximum at 450 nm [10, 17], and neutral tryptophanyl radical Trp$^\bullet$, formed due to rapid deprotonation of the initial cation radical $TrpH^{+\bullet}$, has two maxima at 330 and 510 nm [10, 15, 16]. In the present work the monitoring of the solvated electron decay was performed at the wavelength of 560 nm, where the absorption of e_{aq}^- is much stronger than that of the other intermediates. The triplet decay was measured at 450 nm. The absorption coefficient of Trp$^\bullet$ at 330 nm is approximately 1.5 times higher than that at 510 nm [15]. However, at 330 nm other radical species, such as electron adducts to tryptophan (maximum at 350 nm) or to nucleotides, may contribute to the observed absorbance. Thus, the decay of the Trp$^\bullet$ radical was measured at 510 nm, whereas the formation and evolution of electron adducts were monitored at 350 nm.

In the absence of nucleotides, the rate of the solvated electron decay is proportional to the initial tryptophan concentration $k_{obs} = k_{e\text{-}Trp} \cdot [TrpH]$, $k_{e\text{-}Trp} = 3.0 \times 10^8$ $M^{-1}s^{-1}$, which corresponds to the reaction of electron addition to the ground-state tryptophan. In the presence of AMP, CMP, GMP or UMP the electron decay significantly accelerates due to the formation of electron adducts with nucleotides: $k_{e\text{-}AMP} = 4.0 \cdot 10^9$ $M^{-1}s^{-1}$ $k_{e\text{-}CMP} = 6.8 \cdot 10^9$ $M^{-1}s^{-1}$, $k_{e\text{-}GMP} = 1.5 \cdot 10^9$ $M^{-1}s^{-1}$, and $k_{e\text{-}UMP} = 1.0 \cdot 10^9$ $M^{-1}s^{-1}$ [28]. However, noticeable spectral changes in the region of 300–400 nm were observed only in the photolysis of TrpH with AMP.

Figure 2 demonstrates the absorption kinetics observed after flash photolysis of 5.3 mM TrpH (trace a, in the absence of nucleotides; trace b, in the presence of 5 mM AMP; trace c, in the presence of 5 mM AMP and $5.1 \cdot 10^{-2}$ M acetone). The growth of the absorption observed in the presence of AMP (Fig. 2, trace b) is characterized by the first-order rate constant $(1.7 \pm 0.5) \cdot 10^5$ s^{-1}, which does not depend on the initial TrpH and AMP concentrations and on the intensity of the laser irradiation. Since in our experimental conditions the addition of solvated electrons to AMP should complete within a few nanoseconds, the ob-

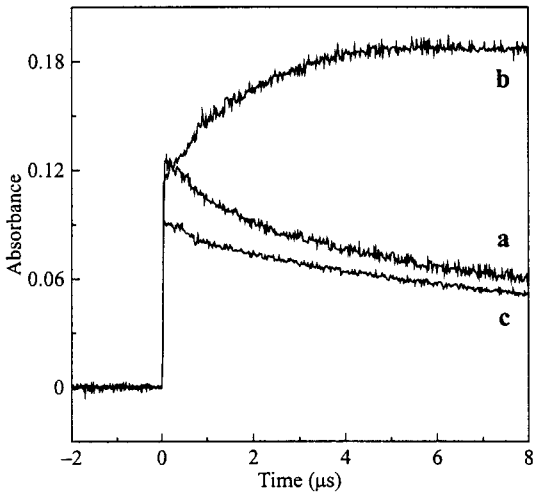

Fig. 2. Transient absorption kinetics obtained during the photolysis of 5.3 mM TrpH (**a**); 5.3 mM TrpH in the presence of 5 mM AMP (**b**); 5.3 mM TrpH in the presence of 5 mM AMP and $5.1 \cdot 10^{-2}$ Macetone at pH 7.0 (**c**).

served growth of the absorption (Fig. 2, trace b) should be attributed to the tautomeric transformation of the adduct from the N-protonated to C-protonated form (Fig. 3). Earlier the reaction of tautomeric transformation of the AMP radical was investigated by means of pulse radiolysis [6, 40].

Acetone scavenges solvated electrons with the rate constant $k_{e\text{-}ac} = 6 \cdot 10^9$ $M^{-1}s^{-1}$ [28]. Thus, the disappearance of the growing component of the kinetics in the presence of acetone (Fig. 2, trace c) testifies that the observed kinetics indeed corresponds to the isomerization of the electron adduct to AMP. Electron scavenging by acetone also prevents the formation of an electron adduct to tryptophan, which explains some decrease of the total absorption of the trace c (Fig. 2) in comparison with the trace a (Fig. 2). A similar kinetics was observed when instead of acetone the solvated electrons were scavenged by N_2O in the presence of 0.2 M t-BuOH ($OH^{\cdot}$ scavenger).

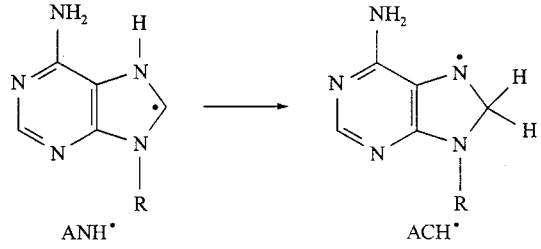

Fig. 3. Reaction of N-protonated radical ($ANH^{\cdot}$) conversion into C-protonated radical ($ACH^{\cdot}$).

Table 1. Rate constants of the triplet tryptophan quenching by nucleotides and the data on CIDNP effects in the photolysis of TrpH with nucleotides.

Nucleotide	$k_q \cdot 10^{-8}$ ($M^{-1}s^{-1}$)	Geminate polarization	F-pair polarization	CIDNP quenching by N_2O
AMP	4.7±1.0	no	yes	complete
CMP	3.1±0.8	yes	yes	complete
GMP	0.56±0.14	no	yes	complete
UMP	5.7±1.2	yes	yes	incomplete

All measurements of the triplet tryptophan quenching by nucleotides were performed in the presence of $(3–4) \cdot 10^{-2}$ M acetone in order to avoid complications connected with the electron adduct formation. The pseudo-first-order triplet quenching rate constants $k_1 = [\text{Nuc}] \cdot k_q$ were measured at 450 nm by decay of the triplet tryptophan signal in the presence of nucleotides, the concentration of nucleotides [Nuc] varied from 10^{-3} to $2 \cdot 10^{-2}$ M. For all four nucleotides, AMP, CMP, GMP, and UMP, a linear dependence of k_1 on the nucleotide concentration has been found. The obtained values of k_q are presented in Table 1.

The triplet energy of tryptophan of 296 kJ/mol [41] is significantly lower than that of nucleotides: 314 kJ/mol for AMP, 321 kJ/mol for CMP, 317 kJ/mol for GMP, and 320 kJ/mol for UMP [42], so the observed quenching cannot be attributed to the triplet energy transfer. The obtained quenching rate constants for three nucleotides, AMP, CMP, and UMP, are of the same order of $(3–6) \cdot 10^8$ $M^{-1}s^{-1}$, whereas for GMP this value is about one order of magnitude lower. Most likely, this relation corresponds to the difference in one-electron reduction potentials for the nucleotides under study. At pH 7 the reduction potentials E_m for adenine, cytosine, and uracil are 1.32, 1.44, and 1.34 respectively, whereas for guanine it is significantly lower, $E_m = 1.04$ V [35]. Thus, we can presume that the triplet tryptophan quenching by nucleotides is associated with the electron transfer from the triplet tryptophan to the nucleotide.

Previous studies have shown that the quantum yield of the tryptophan monophotonic ionization is about 0.04 [21, 23, 24, 43], and the triplet quantum yield is 0.07–0.1 [18, 24]. Thus, if the triplet tryptophan quenching by nucleotides proceeds via the electron transfer from TrpH to the nucleotide, one can expect a significant increase of the $Trp^{\bullet}$ concentration in the presence of nucleotides. However, the addition of AMP, CMP and GMP up to 10^{-2} M did not result in an increase of the $Trp^{\bullet}$ absorption monitored at 510 nm. In fact, even some decrease of the signal intensity has been observed. A similar decrease in the signal intensity was detected when instead of nucleotides the triplet quencher acrylamide (the rate constant of the triplet tryptophan quenching by acrylamide is $k_{T\text{-}AA} = (1.3 \pm 0.2) \cdot 10^9$ $M^{-1}s^{-1}$ [24]) was added to the tryptophan solution.

It is known [17] that ground-state tryptophan quenches its triplet state with the rate constant $k_{T\text{-}Trp} = 1.2 \cdot 10^7$ $M^{-1}s^{-1}$ [24]. The most probable mechanism

of the quenching is the electron transfer, and in the absence of other triplet quenchers tryptophanyl radicals originate from two sources, direct photoionization and triplet-state quenching by ground-state tryptophan. Quenching of triplet tryptophan by acrylamide, AMP, CMP or GMP closes the triplet channel of the radical formation, which results in some decrease of the absorption signal at 510 nm. From this result it is clear that the reaction between triplet tryptophan and three nucleotides, AMP, CMP and GMP, does not lead to the Trp$^{\bullet}$ radical formation. The quenching mechanism probably includes the formation of an excited complex followed by the restoration of ground-state TrpH and a nucleotide.

In the presence of 5 mM UMP a minor (about 15%) increase of the absorption at 510 nm has been detected. This could indicate that at least partly the triplet tryptophan quenching by UMP proceeds with the Trp$^{\bullet}$ formation. In order to investigate this reaction in more detail, the experiments in which the triplet tryptophan yield was enhanced by addition of KBr into solution were performed. It has been shown by Volkert et al. [17] that an interaction between the fluorescent state of photoexcited tryptophan and Br^- ions significantly promotes the formation of the triplet state. The goal of our preliminary experiments was to determine the efficiency of this process. The TrpH solution was irradiated in the presence of 0.25–2 M KBr, the triplet yield was measured by the triplet absorption at 450 nm. All measurements were performed with laser intensities below 10^{10} W/m^2 in order to avoid biphotonic processes [21]. In the absence of Br^- the triplet quantum yield is about 0.07 [24]. By addition of KBr, the triplet yield increases linearly with the Br^- concentration [Br^-]: $T_{Br}/T_0 = 1 + K_{Br} \cdot [Br^-]$, where T_{Br} and T_0 are the triplet yields in the presence and absence of Br^-. The value of the enhancement constant $K_{Br} = 3.0 \pm 0.3$ M^{-1} has been obtained from the plot of the triplet yield over the Br^- concentration.

The experiments on ^{T}TrpH quenching by nucleotides were performed in the following way. A nucleotide (AMP, CMP, GMP, or UMP) or acrylamide (AA) was added into solution of $6.1 \cdot 10^{-3}$ M TrpH in such concentrations that the pseudo-first-order rate constants of the triplet quenching in all five cases were similar and sufficiently high to prevent the radical formation in the reaction of ^{T}TrpH with ground-state TrpH: [AMP] = 6.7 mM, [CMP] = 10.0 mM, [GMP] = 24 mM, [UMP] = 5.5 mM, [AA] = 2.4 mM. The solutions were irradiated in the presence of KBr, and the dependence of the Trp$^{\bullet}$ yield on the Br^- concentration was measured. As expected, for acrylamide and for three nucleotides, AMP, CMP and GMP, no noticeable changes in Trp$^{\bullet}$ absorption have been detected with [Br^-] concentrations of up to 2 M. For UMP a linear growth of Trp$^{\bullet}$ yield with the Br^- concentration was observed (Fig. 4).

The obtained results allow one to estimate the efficiency of the radical formation in the ^{T}TrpH quenching by UMP. With bromide concentration increase from 0 to 2 M the triplet yield increases almost 7-fold from 0.07 to about 0.45, whereas the radical yield grows from 0.04 to 0.09. Since the main channel of the ^{T}TrpH decay is the quenching by UMP, the radical quantum yield in the quenching can be estimated as approximately 0.13.

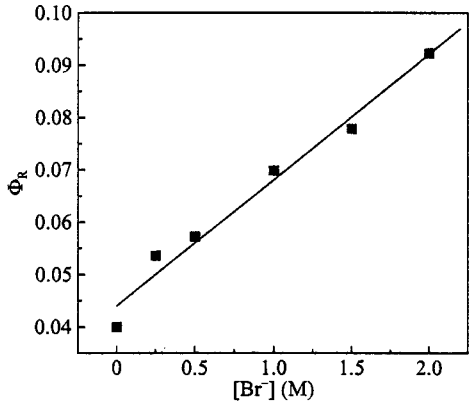

Fig. 4. Dependence of the Trp• quantum yield on the Br^- concentration in the photolysis of $6.1 \cdot 10^{-3}$ M TrpH with $5.5 \cdot 10^{-3}$ M UMP at pH 7.0. Solid line shows the linear fit.

3.2 CIDNP Measurements

The CIDNP measurements confirm the results obtained by the LFP method. CIDNP spectra taken after irradiation of TrpH-nucleotide solutions at pH 7.0 are shown in Fig. 5. All solutions were bubbled with argon before irradiation. The spectra show rather weak polarization on tryptophan protons, assigned to the H_2, H_4, and H_6 protons of the indole ring and to β-protons. The polarization of the H_6 protons of UMP and CMP nucleotides are emissive, and for CMP a weak absorption signal attributed to the H_5 proton was detected. For GMP the polarization was observed only on the H_8 proton, and for AMP the positive polarization on the H_2 and H_8 protons was detected as well as a polarized signal at 4.8 ppm, denoted as H_8(ANH-Trp) in Fig. 6. This signal was assigned to the H_8 proton of the product of the recombination of ANH• and Trp• radicals (Fig. 6).

Earlier the CIDNP data obtained in the stationary photolysis of riboflavin with nucleotides have been published [44]. The polarization for purine nucleotides reported in the present work is in agreement with these data. However, McCord et al. [44] observed the polarization only for pyrimidine bases (cytosine and uracil), whereas for nucleotides CMP and UMP no polarization has been detected. In our experiments strong emissive polarization was observed for both CMP and UMP (Fig. 5).

When TrpH-nucleotide solutions were bubbled with N_2O instead of Ar, the polarization intensity for UMP was significantly reduced, and for the other three nucleotides it disappeared completely. This result testifies that the polarization on AMP, CMP, GMP, and in a large extent on UMP is formed in reactions of electron adducts.

Thus, the following general scheme of CIDNP formation in the photolysis of TrpH with nucleotides (Nuc) can be suggested:

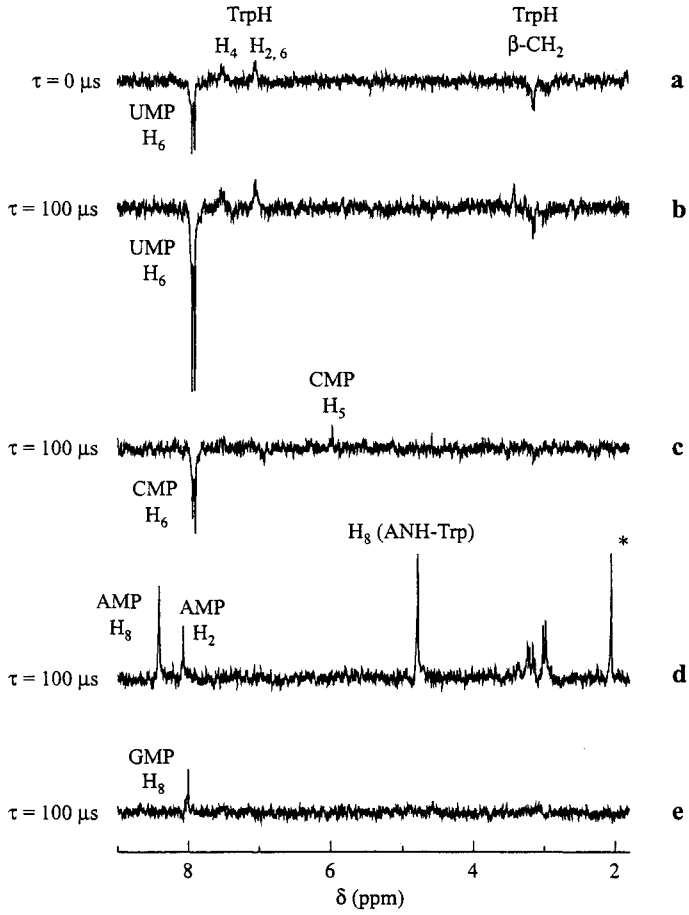

Fig. 5. ^{1}H CIDNP spectra obtained during the photolysis of TrpH with nucleotides: **a** and **b** 10^{-2} M TrpH and $7.7 \cdot 10^{-3}$ M UMP taken immediately and 100 μs after the laser pulse; **c** $1.1 \cdot 10^{-2}$ M TrpH and $1.2 \cdot 10^{-2}$ M CMP, 100 μs after the laser pulse; **d** 10^{-2} M TrpH and $7.5 \cdot 10^{-3}$ M AMP, 100 μs after the laser pulse; **e** $1.2 \cdot 10^{-2}$ M TrpH and $2.1 \cdot 10^{-2}$ M GMP, 100 μs after the laser pulse. For signal assignment, see Figs. 1 and 6. Asterisk denotes an unindentified product.

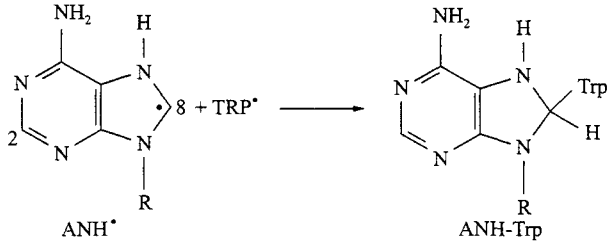

Fig. 6. Recombination reaction of ANH• and Trp• radicals.

$$TrpH \xrightarrow{h\nu} TrpH^{+\bullet} + e^-_{aq}, \quad (1)$$

$$Nuc + e^-_{aq} \longrightarrow Nuc^{-\bullet}, \quad (2)$$

$$TrpH^{+\bullet} \rightleftharpoons Trp^{\bullet} + H^+, \quad (3)$$

$$Nuc^{-\bullet} + H^+ \rightleftharpoons NucH^{\bullet}, \quad (4)$$

$$Trp^{\bullet} + NucH^{\bullet} \text{ (or } Nuc^{-\bullet}) \longrightarrow {}^{*}TrpH, {}^{*}Nuc, {}^{*}Trp\text{-}Nuc. \quad (5)$$

Here the asterisk denotes nuclear polarization.

Generally speaking, the following processes govern the formation of CIDNP effects. Geminate nuclear polarization, observed immediately after the laser pulse, is formed in the products of recombination of geminate radical pairs. The escaped radicals carry the polarization of opposite sign and transfer this polarization to diamagnetic products in bulk reactions. An additional polarization can be formed in random radical encounters in the bulk (F-pairs). The CIDNP formation is also affected by the nuclear paramagnetic relaxation and reactions of degenerate electron or hydrogen exchange [45, 46].

The reaction schemes (1)–(5) suggest that in the photolysis of TrpH with nucleotides the nuclear polarization is formed only in F-pairs (reaction (5)). For purine nucleotides AMP and GMP this suggestion is confirmed by TR-CIDNP measurements: no nuclear polarization has been observed immediately after the laser pulse. In the time range from 0 to about 100 μs the polarization intensity gradually increases, reflecting the CIDNP formation in F-pairs (see Table 1). It is important to note that the geminate polarization has not been detected even for TrpH protons, indicating that no CIDNP is formed in the pair of cation $TrpH^{+\bullet}$ and solvated electron e^-_{aq}. This finding is in agreement with the observation [20, 22] that in the photolysis of aqueous tryptophan the absorption of the solvated electron does not change in the time range from 200 fs to 2 ns, and no geminate recombination in the pair $[TrpH^{+\bullet}\ e^-_{aq}]$ occurs.

For UMP the formation of both geminate and F-pair polarization was observed. Figure 7 demonstrates the CIDNP kinetics obtained for the H_6 protons of UMP in the photolysis of 10^{-2} M TrpH with $7.7 \cdot 10^{-3}$ M UMP at pH 7. The signal intensity for the first experimental point is scaled to unity. The initial signal observed immediately after the laser pulse should be attributed to the polarization formed in the geminate pair of $TrpH^{+\bullet}$ and $UMP^{-\bullet}$ radicals:

$$Trp \xrightarrow{h\nu,\,ISC} {}^{T}TrpH,$$

$${}^{T}TrpH + UMP \longrightarrow {}^{T}[TrpH^{+\bullet}\ UMP^{-\bullet}] \longrightarrow {}^{*}TrpH, {}^{*}UMP.$$

Thus, in the photolysis of TrpH with UMP CIDNP is created by two channels: (i) the triplet tryptophan quenching by UMP yields the geminate radical pair $^{T}[TrpH^{+\bullet}\ UMP^{-\bullet}]$ and (ii) the tryptophan photoionization leads to the formation

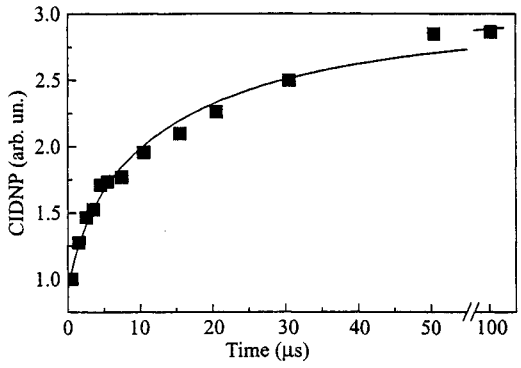

Fig. 7. ^{1}H CIDNP kinetics obtained during the photolysis of 10^{-2} M TrpH and $7.7 \cdot 10^{-3}$ M UMP at pH 7 for H_6 protons of UMP. Solid line shows calculations according to reaction schemes (6)–(8) with parameters: $k_t = 2 \cdot 10^9$ $M^{-1}s^{-1}$, $R_0 = 3.2 \cdot 10^{-5}$ M, $T_1 = 20$ μs, $\gamma = 4.1$.

of electron adducts to UMP and TrpH, bulk reactions of these radicals result in the CIDNP formation in F-pairs. The CIDNP kinetics was simulated by the approach introduced by Vollenweider et al. [47]. The following equations describe the time dependence of the radical concentration R and the polarization of the radicals $P(\mathrm{R})$ and the products $P(\mathrm{Pr})$:

$$R(t) = \frac{R_0}{1 + k_t R_0 t}, \tag{6}$$

$$\frac{dP(\mathrm{R})}{dt} = -k_t P(\mathrm{R}) R - k_t \beta R^2 - \frac{P(\mathrm{R})}{T_1}, \tag{7}$$

$$\frac{dP(\mathrm{Pr})}{dt} = k_t P(\mathrm{R}) R + k_t \beta R^2. \tag{8}$$

Here k_t is the radical termination rate constant; its value was determined by LFP measurements of the radical decay, and is equal to $2 \cdot 10^9$ $M^{-1}s^{-1}$. R_0 is the initial radical concentration, T_1 is the nuclear paramagnetic relaxation time. The parameter β represents the polarization per radical pair, created in F-pairs; it is related to the geminate polarization P^G via quantity γ [48], which is the ratio of polarizations created in F- and geminate pairs:

$$\beta = \gamma P^G / R_0.$$

In Eqs. (7) and (8) the first term describes the polarization transfer from the radicals to ground-state molecules in the termination reaction, the second term represents the formation of polarization in F-pairs. The third term in Eq. (7) corresponds to the loss of polarization in the radicals due to nuclear relaxation. It is assumed that the yield of radicals that escape from the triplet radical pair is much greater than the yield of geminate recombination, and that the radicals decay only

in the cross-termination reaction. The initial polarizations are taken as $P(Pr)_0 = P^G = -P(R)_0$. Thus, in the simulation of CIDNP kinetics (Fig. 7, solid line) there were three fitting parameters, R_0, T_1, and γ. The best fit was obtained with $R_0 = 3.2 \cdot 10^{-5}$ M, $T_1 = 20$ ms, and $\gamma = 4.1$. A similar value for T_1 has been recently reported for the H_8 proton of GMP [49]. The essentially short nuclear relaxation times for radicals of nucleotides should be probably attributed to a high anisotropy of hyperfine interaction constants in these radicals. The obtained value $\gamma = 4.1$ is unusually high, in the typical case when radicals are generated only in geminate processes this value is expected to be 2.7–2.9 [47]. The high value of γ reflects the fact that the polarization in the bulk is formed not only by radicals which escaped the geminate recombination but also by electron adducts to UMP.

Unexpectedly, the presence of the geminate polarization has been also observed in the photolysis of TrpH with CMP, although the contribution of the geminate polarization in the case of CMP is smaller than in the case of UMP. LFP measurements have not revealed the radical formation in the triplet TrpH quenching by CMP, but the results of the TR-CIDNP experiments probably indicate that some minor radical formation takes place.

Acknowledgements

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