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Electronic Supplementary Information

Indirect NMR Detection of Transient Guanosyl Radical Protonation in Neutral Aqueous Solution

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Experimental methods and sample preparation

Time-Resolved CIDNP

A detailed description of our TR-CIDNP setup can be found in a previous publication.[1] Briefly, samples in standard 5 mm Pyrex NMR tubes placed in the probe of a Bruker Avance III 400 MHz NMR spectrometer were irradiated by a Coherent Compex Pro 102 XeCl excimer laser (wavelength 308 nm and pulse duration of 15 ns, output pulse energy used was ~80 mJ). The pulse sequence for TR-CIDNP experiments is as follows: radiofrequency (RF) saturation pulses – laser pulse – evolution time τ – RF detection pulse – free induction decay. Since the background signals in the spectrum originating from Boltzmann polarization are suppressed, only resonances from the polarized products formed during the variable delay τ appear in the ^{1}H CIDNP spectra. In all kinetic measurements, an RF pulse with duration of 1 μs was used for detection. To take into account the finite length of the RF-pulse, the time scale was shifted by one half of the RF-pulse duration, i.e. in all plots in Fig. 2 the time of 0.5 μs corresponds to $\tau=0$ μs, 1.5 μs stands for $\tau=1$ μs, etc.

Chemicals

Guanosine-5′-monophosphate (GMP), 3,3′,4,4′-tetracarboxy benzophenone (TCBP), disodium phosphate, DCl (35 wt. % solution in D_2O, 99 + atom % D), NaOD (30% solution in D_2O), and D_2O (99.9% enriched) were used as received from Sigma-Aldrich. The concentration of TCBP was 2 mM, of GMP – 20 mM. The pH of the NMR samples was adjusted by addition of DCl or NaOD.

Simulation of CIDNP kinetics obtained in the photoreaction of TCBP and GMP

To determine the rate of formation of the cation radical $(G^{\bullet +})'$, a simulation of the CIDNP kinetics was performed. The set of equations introduced by Fischer for the cyclic radical reactions[2] was modified to take into account reversible protonation of the neutral guanosyl radical. The peculiarity of the photo-system under study is that for radical pairs containing a TCBP radical the reaction of back electron transfer is possible not only from their singlet correlated state with restoration of the initial compounds, but also can partly proceed from their triplet state,[3] resulting in the formation of TCBP in its lower triplet state and quencher (GMP) in its ground state. Since the singlet recombination prevails (see below), all the considerations given in the main text qialitatively refer to the case of triplet precursor and singlet recombination of a radical pair. However, without taking into account triplet channel of recombination, satisfactory agreement between experimental and simulated CIDNP kinetics could not be achieved.

The concentrations of the radicals are described by the system of algebraic equations:

$$R(t) = \frac{R_0}{1 + k_{\mathrm{t}} R_0 t} \tag{s1}$$

$$R_{\mathrm{N}}(t) = \frac{R_0 e^{-k_{\mathrm{p}} t}}{1 + k_{\mathrm{t}} R_0 t} \tag{s2}$$

$$R_{\mathrm{C}}(t) = \frac{R_0 (1 - e^{-k_{\mathrm{p}} t})}{1 + k_{\mathrm{t}} R_0 t} \tag{s3}$$

where R is the concentration of the $TCBPH^{\bullet}$ radical, R_{N} and R_{C} are the concentrations of neutral radical $G(-H)^{\bullet}$ and cation radical $(G^{\bullet +})'$, respectively; k_{t} is the second-order radical termination rate constant; k_{p} the rate constant of protonation of the neutral guanosyl radical. It is assumed that the fraction of geminate recombination can be neglected, and R_0 is the initial concentration of radicals of each type, which participate in the bulk processes.

The polarization in the neutral radical P_N^R, in the cation radical P_C^R, and in ground-state guanosine P_{N+C} are described by the following equations:

$$\frac{dP_N^R}{dt} = -k_t P_N^R R - k_t \beta_N R R_N + 2\chi_N k_t \beta_N R R_N - \frac{P^R}{T_1^N} - k_p P_N^R \tag{s4}$$

$$\frac{dP_C^R}{dt} = -k_t P_C^R R - k_t \beta_C R R_C + 2\chi_C k_t \beta_C R R_C - \frac{P^R}{T_1^C} + k_p P_N^R \tag{s5}$$

$$\frac{dP_{N+C}}{dt} = k_t P_N^R R_N + k_t P_C^R R + k_t \beta_N R R_N + k_t \beta_C R R_C - 2\chi_N k_t \beta_N R R_N - 2\chi_C k_t \beta_C R R_C \tag{s6}$$

Here, χ_N and χ_C are the probabilities of triplet recombination of the radical pairs of TCBP radical, and neutral or cationic guanosyl radicals, respectively. T_1^N and T_1^C are the relaxation times of proton H8 in the guanosyl radicals. Earlier T_1=20 μs was found for guanosyl radicals in different protonation forms, from the dicationic to the neutral one. The parameters β represent the polarization per pair, created upon recombination of F-pairs of radicals in the bulk, and are related to the geminate polarization P^G via the quantity γ, which is the ratio of polarization created in F-pairs to the geminate polarization: β_N and β_C stand for the radical pairs with neutral and cation radicals, $\beta_N = \gamma_N P^G/R_0$, $\beta_C = \gamma_C P^G/R_0$, with P^G denoting the geminate polarization formed in the pair of TCBPH$^\bullet$ and G(-H)$^\bullet$. Since we do not measure the absolute value of polarization, in the simulation P^G appears as vertical scaling factor, which for convenience of presentation is scaled to unity. The initial polarizations were taken as $P^G = -P_N^R = 1$.

Equations s7-s9 describe nuclear polarization in the TCBP radical, P^R, TCBP in its lower triplet state, P^T, and its ground state TCBP, P^S, which is detected by NMR:

$$\frac{\mathrm{d}P^{\mathrm{R}}}{\mathrm{d}t} = -\,k_{\mathrm{t}}P^{\mathrm{R}}R_{\mathrm{N}} - k_{\mathrm{t}}P^{\mathrm{R}}R_{\mathrm{C}} - k_{\mathrm{t}}\beta_{\mathrm{N}}RR_{\mathrm{N}} - k_{\mathrm{t}}\beta_{\mathrm{C}}RR_{\mathrm{C}} - \frac{P^{\mathrm{R}}}{T_1} \tag{s7}$$

$$\frac{\mathrm{d}P^{\mathrm{T}}}{\mathrm{d}t} = \chi_{\mathrm{N}}k_{\mathrm{t}}P^{\mathrm{R}}R_{\mathrm{N}} + \chi_{\mathrm{C}}k_{\mathrm{t}}P^{\mathrm{R}}R_{\mathrm{C}} + \chi_{\mathrm{N}}k_{\mathrm{t}}\beta_{\mathrm{N}}RR_{\mathrm{N}} + \chi_{\mathrm{C}}k_{\mathrm{t}}\beta_{\mathrm{C}}RR_{\mathrm{C}} - \frac{P^{\mathrm{T}}}{T_1^{\mathrm{T}}} - \frac{P^{\mathrm{T}}}{\tau^{\mathrm{T}}} \tag{s8}$$

$$\frac{\mathrm{d}P^{\mathrm{S}}}{\mathrm{d}t} = (1-\chi_{\mathrm{N}})k_{\mathrm{t}}P^{\mathrm{R}}R_{\mathrm{N}} + (1-\chi_{\mathrm{C}})k_{\mathrm{t}}P^{\mathrm{R}}R_{\mathrm{C}} + (1-\chi_{\mathrm{N}})k_{\mathrm{t}}\beta_{\mathrm{N}}RR_{\mathrm{N}} + (1-\chi_{\mathrm{C}})k_{\mathrm{t}}\beta_{\mathrm{C}}RR_{\mathrm{C}} + \frac{P^{\mathrm{T}}}{\tau^{\mathrm{T}}} \tag{s9}$$

Here, T_1 is the relaxation time of the H6,6′ protons in the TCBPH$^\bullet$ radical, T_1^{T} is the relaxation time of the nuclei in TCBP in its lower triplet state, τ^{T} the lifetime of TCBP in its lower triplet state, while χ_{N} and χ_{C} are the probabilities of radical pair recombination to the TCBP triplet state, involving the neutral or the cationic guanosyl radical, respectively. The initial polarizations (formed during the geminate stage) were taken as $P^{\mathrm{GS}}=-P^{\mathrm{R}}$, $P^{\mathrm{GT}}=0$. The vertical scaling was adjusted to keep the relative intensities of GMP and TCBP signals.

The parameters known from previous investigations were: $T_1^{\mathrm{N}}=T_1^{\mathrm{C}}=20$ μs. In the present simulation it was found that the best agreement between the experimental data and simulation was obtained for $T_1^{\mathrm{N}}=15$ μs, $T_1^{\mathrm{C}}=33$ μs. The parameters known from studies of the CIDNP kinetics of TCBP and amino acids are: $T_1=100$ μs, $T_1^{\mathrm{T}}=100$ μs, and $\tau^{\mathrm{T}}=1$ ms (the corresponding manuscript is under preparation). The fitting parameters were k_{p} and $k_{\mathrm{t}}R_0$. The best-fit was obtained at $\chi_{\mathrm{N}}=0.12$, $\chi_{\mathrm{C}}=0.33$, $\gamma_{\mathrm{N}}=7$, $\gamma_{\mathrm{C}}=-9$. The value of $\gamma_{\mathrm{N}}=7$ which is higher than $\gamma=3$ as is typical for the case when geminate and F-pairs are identical. The enhanced γ_{N} is in accordance with radical pair substitution at the geminate stage, which attenuates the geminate CIDNP intensity to a degree depending on the lifetime of the primary guanosyl radical as compared to the lifetime of the geminate pair.[4] Thus, CIDNP formed in an F-pair is higher than that formed in a geminate pair, hence the value of γ is enlarged. From the simulation of the CIDNP kinetics, obtained in the photoreaction of TCBP and GMP in 50 mM sodium phosphate buffer at pH 6.9, the observed rate constant of protonation of G(-H)$^\bullet$ was determined to be k_p=(1.8±0.4)×10^6 s^{-1}.

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