Electron Transfer in Dipeptides

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Intramolecular Electron Transfer in the Photooxidized Peptides Tyrosine–Histidine and Histidine–Tyrosine: A Time-Resolved CIDNP Study**

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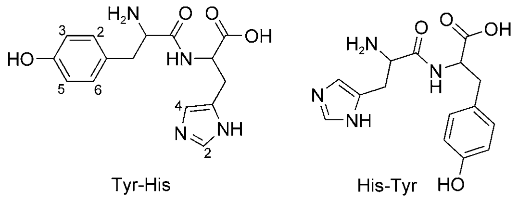
The long-range electron-transfer (ET) reaction with the participation of tyrosyl radicals is known to be of great importance in proteins. A binding partner of tyrosyl radicals at the active sites of several enzymes and in model peptides is histidine.[1] For example, the formation of the remarkably stable tyrosine radical in photosystem II is attributed to proton-coupled electron transfer, during which a proton is relocated to the imidazole group of an adjacent histidine residue.[2] The efficiency of this kind of ET was measured in model peptides containing a photochemically generated electron-acceptor group, N-terminal tyrosine as a donor, and a relay amino acid in between.[3] Indirect evidence for the proton-coupled electron transfer from tyrosine to the hystidyl radical was drawn from the optical detection of tyrosyl radicals when histidine was used as the relay amino acid instead of alanine. However, kinetic information about electron transfer from tyrosine to the histidyl radical has not been obtained so far.

To study the kinetics of intramolecular electron transfer between tyrosine and the histidyl radical, we chose two dipeptides, histidine–tyrosine (His-Tyr) and tyrosine–histidine (Tyr-His) as model systems (Scheme 1). The method of time-resolved chemically induced dynamic nuclear polarization[4,5] (TR CIDNP) used in the present study has the following advantage over conventional pulse radiolysis: the histidine radical is a weak chromophore, and transient absorption measurements restrict observation to the tyrosyl radical,[6] whereas the CIDNP technique enables the reactions of transient histidyl and tyrosyl radicals to be followed by NMR spectroscopic detection of signals of both residues.[7,8]



Scheme 1. Molecular structures of the peptides studied.

TR CIDNP has proved to be a good tool for establishing reaction mechanisms and for the determination of intra- and intermolecular electron-transfer rate constants.[9–11] The nonequilibrium population of nuclear spin states, known as CIDNP, arises from the dependence of the rate of intersystem crossing in a radical pair on the configuration of nuclear spins that have a nonzero hyperfine interaction (HFI) with an unpaired electron and results in anomalous NMR intensities for the nuclei involved.[5,12] The essence of the TR CIDNP method in the investigation of reductive electron-transfer reactions is as follows: The radicals whose reduction is the subject of study are generated by quenching of a photoexcited dye molecule. At the geminate stage of the reaction, which is not resolved in our experiment, the formation of CIDNP is detected as NMR enhancement with no delay after the laser pulse. According to the spin-sorting nature of the S–T_0 mechanism of CIDNP formation, the radicals that escape geminate termination are "marked" by nuclear polarization of the opposite sign to that of the geminate polarization. Upon the reduction of radicals in the bulk, this CIDNP is canceled out by the geminate polarization, and the decay of CIDNP is observed (the so-called CIDNP cancellation effect). The high sensitivity of the observed CIDNP kinetics to the rate of reductive electron transfer permits a quantitative study of this reaction.[9]

Pulse radiolysis studies on the dipeptide His-Tyr provided an estimate of over $10^6\,s^{-1}$ for the rate constant of the intramolecular electron-transfer reaction.[6] However, the authors assumed that the histidyl radical was produced in cationic form, whereas our CIDNP investigation of *N*-acetylhistidine indicated that the histidyl radical at pH 7 exists in its neutral form. Otherwise, degenerate electron exchange with a parent molecule would be expected, a process which was not observed.[7] Thus, in the present study, our observations refer to the neutral radical His$^{\cdot}$ in the dipeptides.

In our experiments, the radicals were formed by quenching of the photoexcited triplet dye [D_8]-2,2′-dipyridyl (DP). We were interested in the photochemical formation of histidyl radicals to study their subsequent reduction by tyrosine. However, it is impossible to avoid the simultaneous formation of tyrosyl radicals. As established previously, histidine

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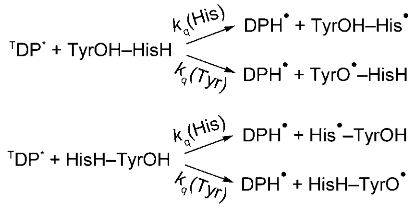
quenches DP through hydrogen-atom transfer with a maximum quenching rate constant of $4\times10^{7}\,\mathrm{M}^{-1}\mathrm{s}^{-1}$ at pH 6.[7] The mechanism of triplet DP quenching by tyrosine is electron transfer with a rate constant of $3.5\times10^{9}\,\mathrm{M}^{-1}\mathrm{s}^{-1}$ at acidic pH values and hydrogen transfer with a rate constant of $7.2\times10^{7}\,\mathrm{M}^{-1}\mathrm{s}^{-1}$ at pH = 7.[8] Thus, at any pH value, the concentration of the radicals generated by triplet quenching (Scheme 2) and therefore the CIDNP signal intensity are higher for tyrosine than for histidine.



Scheme 2. Quenching of triplet excited 2,2′-dipyridyl by the peptides TyrOH–HisH and HisH–TyrOH. "H" in HisH refers to the hydrogen atom attached to the nitrogen atom in the 1-position, and "H" in TyrOH refers to the phenolic hydrogen atom.

We chose to carry out our experiments at pH 7.3. At this pH value, the mechanism of triplet DP quenching by tyrosine is hydrogen transfer, and the histidine signal in the CIDNP spectra was analyzable. The CIDNP spectra obtained for the photoreaction of DP with the peptides Tyr-His and His-Tyr are shown in Figure 1. The CIDNP pattern for each residue resembled that for the corresponding free amino acid: enhancements were observed for 2-H, 4-H, and β-CH_2 of His, and 2,6-H, 3,5-H, and β-CH_2 of Tyr; polarization signs were in accordance with the Kaptein rules.[13]

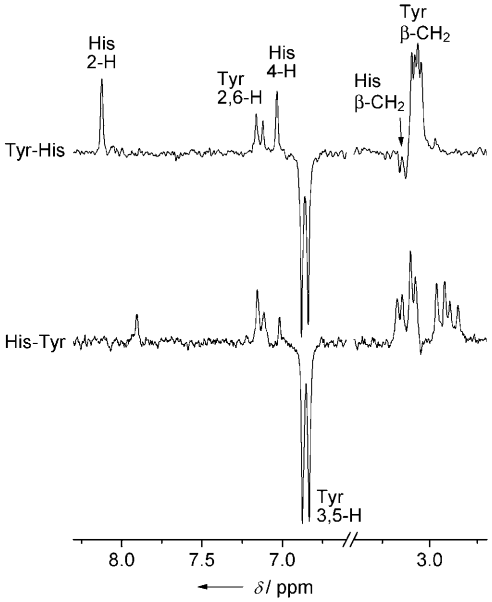


Figure 1. ¹H NMR CIDNP spectra (200 MHz) recorded for the photoreaction between 2,2′-dipyridyl and the peptides Tyr-His (top spectrum) and His-Tyr (bottom spectrum) at pH 7.3 immediately after the laser pulse.

The CIDNP kinetics of 2-H of the His residue are shown in Figure 2a. The polarization decay is typical of reductive electron transfer to the radical whose product is observed.[9–11] For comparison, in the present study, we determined the kinetics of the photoreaction between DP and the peptide histidine–phenylalanine. The CIDNP of 2-H of the histidine residue in His-Phe was observed to increase with time (stars in Figure 2b). Thus, the CIDNP decay can be attributed to intramolecular electron transfer from the tyrosine residue to the histidyl radical. On the basis of our observations, we can not discriminate between the following reaction mechanisms: electron transfer followed by protonation of the histidine anion and deprotonation of the tyrosine cation radical, or proton-coupled electron transfer. The general reaction is described by Equation (1a,b):

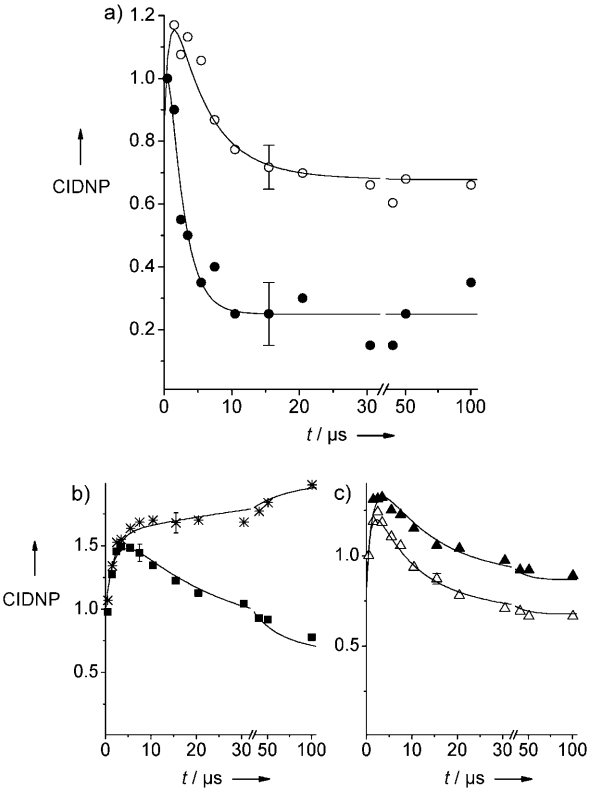


Figure 2. a) ¹H CIDNP kinetics found for the 2-H atom of the histidine residue in His-Tyr (solid circles) and Tyr-His (open circles) during the photoreaction between 2,2′-dipyridyl and the corresponding dipeptide at pH 7.3. b) ¹H CIDNP kinetics found for the 2-H atom (stars) and the β-hydrogen atoms (squares) of the histidine residue in His-Phe during the photoreaction between 2,2′-dipyridyl and His-Phe at pH 6.8. c) ¹H CIDNP kinetics found for the 3,5-H atoms of the tyrosine residue in His-Tyr (solid triangles) and Tyr-His (open triangles) during the photoreaction between 2,2′-dipyridyl and the corresponding dipeptide at pH 7.3. CIDNP values are given in normalized units.

$$\mathrm{TyrOH{-}His^{\bullet}} \xrightarrow{k_e} \mathrm{TyrO^{\bullet}{-}HisH} \tag{1a}$$

$$\mathrm{His^{\bullet}{-}TyrOH} \xrightarrow{k_e} \mathrm{HisH{-}TyrO^{\bullet}} \tag{1b}$$

The observed CIDNP kinetics depends not only on the rate constant of reaction (1a) or (1b), but also on a number of other parameters, such as the second-order radical-decay rate and nuclear paramagnetic relaxation time. To determine the rate constants for reactions (1a) and (1b) for both oxidized peptides, we simulated the CIDNP kinetics as we had done previously for the Trp-Tyr peptide[9] and for the reduction of the guanosyl radical by amino acids.[10,11] The simulation was based on the approach of Vollenweider and Fischer[14] and modified to take into account reaction (1a) or (1b). A comprehensive description of the simulation procedure is available as Supporting Information.

To reduce the number of fitting parameters in the simulation procedure, we assumed that the paramagnetic nuclear relaxation time for the 2-H proton of the His residue is determined by molecular rotation, and that the HFI anisotropy is approximately the same for all three peptides. The CIDNP kinetics found for 2-H of His-Phe is not complicated by an electron-transfer reaction, and T_1 could be extracted with high accuracy. To improve the accuracy, we simulated the CIDNP kinetics for 2-H together with that of the β-hydrogen atoms in His-Phe by using a common parameter $R_0 k_t$. We used an additional constraint: the ratio of relaxation times for 2-H and the β-hydrogen atoms was fixed to the value for the radical derived from *N*-acetylhistidine, that is, 16:200, which is valid when the difference in relaxation times between the peptide radical and the radical of the *N*-acetyl derivative is determined by the difference in motional properties only. By applying the described simulation procedure, we obtained the value $T_1 = (13 \pm 3)$ μs for 2-H (163 μs for the β-hydrogen atoms). For the tyrosyl radical in the peptides under study we assume that T_1 is reduced by the same factor as for the His residue. This assumption gives $T_1 = 63 \times 13/16 = 51$ μs; $T_1 = 63$ μs was determined for the *N*-acetyltyrosyl radical.[8]

Since the CIDNP kinetics of His is more sensitive to the rate constant k_e, whereas the kinetics of Tyr (Figure 2c) is more sensitive to the parameter of the second-order termination reaction, $R_0 k_t$, we performed a simultaneous simulation of the kinetics of both the 2-H atom of His and 3,5-H of Tyr. The fitting parameters were $R_0 k_t$, k_e, and the scaling factors for CIDNP intensity. The best-fit simulations are shown in Figure 2a,c by solid lines. The best-fit values of $R_0 k_t$ are 4.3×10^5 s^{-1} for Tyr-His and 2.9×10^5 s^{-1} for His-Tyr.

The rate constants of intramolecular electron transfer are: $(7.5 \pm 1.5) \times 10^4$ s^{-1} and $(3.8 \pm 0.8) \times 10^5$ s^{-1} for the oxidized peptides Tyr-His and His-Tyr, respectively. The higher k_e value obtained for His-Tyr may be a result of the shorter distance between the residues in this peptide in comparison with that in Tyr-His. Alternatively, it can be explained by the influence of the positive charge of the terminal amino group. A similar difference in k_e values was found for dipeptides Tyr-Trp and Trp-Tyr.[15]

In conclusion, we have demonstrated that the oxidized peptides Tyr-His and His-Tyr with the radical center at the His residue undergo intramolecular electron transfer, which leads to the formation of peptide radicals with the radical center at the Tyr residue. This process was shown unambiguously by the decay of CIDNP kinetics detected for the His residue in both peptides, in contrast with observations for the His-Phe peptide, in which no electron-transfer reaction takes place. We are currently studying the pH-value dependence of the intramolecular electron-transfer reaction in oxidized dipeptides composed of Tyr and His.

Experimental Section

Our setup for TR CIDNP detection has been described in detail previously.[10,16] The samples, sealed in a standard 5 mm Pyrex NMR tube, were irradiated by a COMPEX Lambda Physik XeCl excimer laser in the probe of a 200 MHz Bruker DPX-200 NMR spectrometer. The following pulse sequence was used for TR CIDNP experiments: radiofrequency (RF) saturation pulses—laser pulse—evolution time τ—radiofrequency detection pulse—free induction decay. In all kinetic measurements, an RF pulse with a duration of 1 μs was used for detection. The timing corresponds to the center of the RF pulse (i.e. 0.5 μs for $\tau = 0$) on all CIDNP plots in Figure 2. The pH value of the NMR samples was adjusted by the addition of DCl or NaOD. The dipeptides His-Tyr, Tyr-His, and His-Phe we purchased from Bachem and used without further purification; DCl, NaOD (30% solution in D_2O), and D_2O (99.9% enriched) were used as received from Sigma–Aldrich; [D_8]-2,2′-dipyridyl was purchased from from CDN Isotopes. [D_8]Dipyridyl was used to avoid signal overlapping. The dipeptides were used at a concentration of 30 mM; DP was used at a concentration of 8 mM. No correction was made for the deuterium-isotope effect on the pH value.

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