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Photo-CIDNP Study of the Interaction of Tyrosine with Nifedipine. An Attempt to Model the Binding Between Calcium Receptor and Calcium Antagonist Nifedipine¶

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ABSTRACT

This article proposes a new approach to the modeling of the molecular-level mechanism of ligand–receptor interaction for Ca^{2+} receptor binding site. Chemically induced dynamic nuclear polarization (CIDNP) technique has been used to unravel fine details of the reaction in the model system composed of one of the known Ca^{2+} antagonist drugs, nifedipine (NF), and isolated amino acid residuals (*e.g.* tyrosine [Tyr]) of Ca^{2+} receptor binding site. It has been conclusively demonstrated that the reaction between NF and Tyr resulting in the oxidation product—nitroso form of NF—obeys the radical mechanism. CIDNP data in combination with the results of mathematical modeling of the structures of ligand–receptor complexes have allowed to propose the mechanism of the interaction of NF with Ca^{2+} receptor binding site.

INTRODUCTION

The understanding of the mechanisms of action of a drug at the molecular level is an important objective of biochemistry and pharmacology. If successful, it not only will have a fundamental significance but also will provide for the understanding of the mechanisms of ligand–receptor interactions that might become the determinant in the development of new drug preparations. The knowledge of the fine details of a process defining the binding and dissociation of the receptor complex with a drug will allow to understand the cause of therapeutic action, to propose the methods to control the action of the drug and to minimize possible side effects.

The binding is thought to be a key step of the formation of the complex between the drug and receptor protein. At present, the physicochemical aspects of the binding, however, have not received an appropriate study. Only recently has the issue of molecular-level reaction mechanisms in the active site of cell-membrane receptor (1) started to come to the forefront, gradually replacing the widely used macroscopic approach. In particular, the reference data lack the clarification for what forces the given drug to bind the definite receptors and whether in that case any transformations of the drug molecule occur, stipulating further dissociation of drug–receptor complex.

This article proposes the model describing the binding of the known Ca^{2+} antagonist nifedipine (NF) with Ca^{2+} receptor, which potentially could be extended onto the process in the living organisms. In the frames of this hypothetical model, we propose possible answers to the questions posed above. Underlying the model is the suggestion of the existence of redox interactions of the drug with the receptor site elements, resulting in the chemical transformation of the drug and, thus, ensuring the reversibility of binding (dissociation of the drug–receptor complex). The model is formulated based on the investigation of the mechanisms of the reaction in solution between NF and tyrosine (Tyr), one of the amino acids forming the binding site of Ca^{2+} receptor.

The mechanisms of chemical interaction between NF and Tyr is studied by means of nuclear magnetic resonance (NMR) and chemically induced dynamic nuclear polarization (CIDNP) technique including the time-resolved version. CIDNP techniques allow to detect the active short-lived intermediates and to reconstruct the detailed scheme of the process. The assumptions concerning the processes that occur within the ligand–receptor complexes are made based on the analysis of physicochemical studies of redox interaction between NF and Tyr in solution and the results of the modeling of possible structures of the complex of receptor protein with NF and the product of its transformations.

MATERIALS AND METHODS

Chemicals. NF (1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid dimethyl ester) was a kind gift from Dr. V. Lusis (Institute of Organic Synthesis, Latvian Academy of Sciences, Riga, Latvia). Deuterated solvents D_2O, CD_3OD and CD_3CN (Aldrich) and *N*-acetyl Tyr (Sigma), as well as triphenylamine (TPA, Fluka), were used as supplied. The solutions (concentrations of reagents were in the range of 10^{-3} to 10^{-2} *M*) were deaerated by Ar bubbling.

Apparatus. In CIDNP experiments, the samples in standard 5 mm Pyrex NMR tubes were irradiated directly in the probe of the NMR spectrometer at room temperature. EMG 101 MSC Lambda Physik excimer laser was used as the light source ($\lambda = 308$ nm; pulse duration, 15 ns; average pulse energy, 100 mJ). In the photochemical reaction, CIDNP spectra were detected using DPX 200 Bruker NMR spectrometer (200 MHz ^1H operating frequency). In the time-resolved chemically induced dynamic nuclear polarization (TR CIDNP) experiments, we used the standard presaturation technique to suppress the equilibrium signals involving the sequence: (1) saturating radiofrequency pulse; (2) laser pulse; (3) time delay; (4) detecting

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Abbreviations: CIDNP, chemically induced dynamic nuclear polarization; ET, electron transfer; HFI, hyperfine interaction; NF, nifedipine; NMR, nuclear magnetic resonance; NONF, nitroso form of nifedipine; TPA, triphenylamine; TR CIDNP, time-resolved chemically induced dynamic nuclear polarization; Tyr, tyrosine.

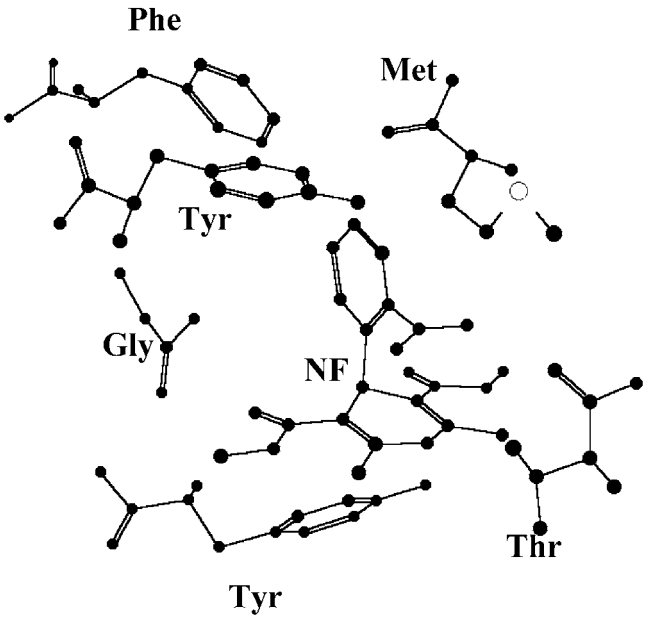


Figure 1. Schematic Chem3D Pro (Cambridge Software, Cambridge, MA) presentation of NF bound to Ca^{2+} receptor active site formed by amino acid residues. Conventional notation used (Tyr, tyrosine; Phe, phenylalanine; Gly, glycine; Thr, threonine; Met, methionine). Data necessary to create this structure were partially taken from Schleifer (2); MM2 procedure has been used for energy minimization.

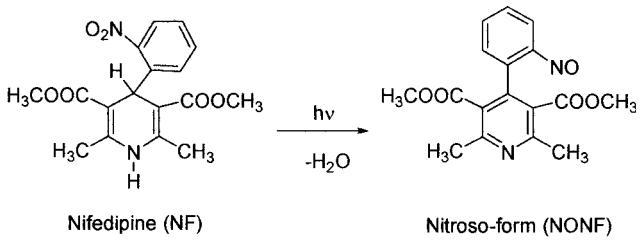
radiofrequency pulse; and (5) free induction decay. In the TR CIDNP experiments, a 1 μs detecting radiofrequency pulse was used, which is approximately equivalent to a 15° pulse. Because the background (equilibrium) NMR signals in CIDNP spectrum were suppressed, only the signals of the products demonstrating nuclear polarization could be observed.

RESULTS

General note

It is well known that the active sites of many cell receptors comprised a set of amino acid residues, each playing a certain role in the binding and dissociation of the ligand–receptor complex. For instance, the active site of one of the most studied calcium channel receptors is formed by six amino acid residues, and the spatial structure of this site changes for open and closed channel (2). Precisely, these changes of spatial structure defining the channel status form the basis of the therapeutic effect of the calcium antagonists widely used in cardiology (*e.g.* antiarrythmics).

According to current understanding, one of the most used dihydropyridine drugs—NF—forms the complex with Ca^{2+} receptor active site with the spatial distribution corresponding to the closed channel (2). Thus, bonded NF precludes the variation in amino acid positions, which in turn signals the channel opening. The dihydropyridine cycle bonds to the three amino acids of the active site that stabilize the complex, whereas the nitrophenyl substituent located out of plane and rotated at a right angle to this plane enters donor–acceptor interaction with another three amino acids, first of all with Tyr (Fig. 1). However, the mechanism of further transformations at the molecular level is unclear. What reaction is underlying the dissociation of the complex and the release of the receptor—after all NF provides only reversible inhibition of Ca^{2+} inflow to the cell?



Scheme 1. Phototransformations of NF.

Note that among known Ca^{2+} antagonists, NF is the very convenient chemical to answer the above question because its transformations in the reaction with various agents as well as under UV irradiation were actively studied both *in vivo* and *in vitro* (1,3,4). One might point out two problems that have been put in the focus of the majority of investigations. First, it is the photolability of the drug, which could potentially lead to the phototransformations of NF *in situ* in the living organism (1). Another problem is stipulated by the nitro group present in the NF molecule because the partial reduction of $-NO_2$ is believed to be the main reason of the most adverse side effects when receiving NO_2-containing drugs. The metabolism of NF by liver cytochrome P-450 has been studied in detail (5), and its main product is, indeed, the corresponding nitropyridine.

It is also known that under UV irradiation, NF is quantitatively converted to the nitroso form of nifedipine (NONF, 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester, Scheme 1) (3). At the same time, NONF is also known to be one of the short-lived intermediates detected under UV irradiation in the living systems (4,6).

As has been mentioned above, at present, literature lacks data on the possible chemical transformation of NF on reaction with Tyr, which is the amino acid residue closest to the nitrophenyl group of NF blocking Ca^{2+} receptor (see Fig. 1). From our viewpoint, the electron transfer (ET) might be potentially the most probable mechanism of the interaction of NF with the environment of the prosthetic group of Ca^{2+} receptor because the detailed theoretical studies provide the evidence of the noticeable charge transfer between NF and Tyr residual in the receptor's binding site (2,7). It is our suggestion that the interaction between NF and Tyr could involve an elementary step of the single-electron transfer. In addition to the above theoretical considerations, this assumption has a number of experimental grounds because NF molecule incorporates both the nitrophenyl and dihydropyridine functions, whereas the nitroaromatics (8,9) and dihydropyridine ring (10–12) have known to enter the single-electron transfer reactions with electron donors and acceptors, respectively.

This article attempts to satisfy the question of the chemical interactions that might occur between a drug and an amino acid residue in the active site of a cell receptor and how these interactions might affect ligand–receptor binding. To answer the above question we will try use the model process. As such, we have selected the reaction of NF with Tyr in solution under UV irradiation. The proposed approach also follows the postulate that the reactivity and reaction mechanisms of paramagnetic species are independent of the methods of their generation. This modeling is necessary because the simple mixing of NF with amino acids, in particular, with Tyr in homogeneous solution does not result in any chemical interaction. Therefore, the use of the photo-CIDNP

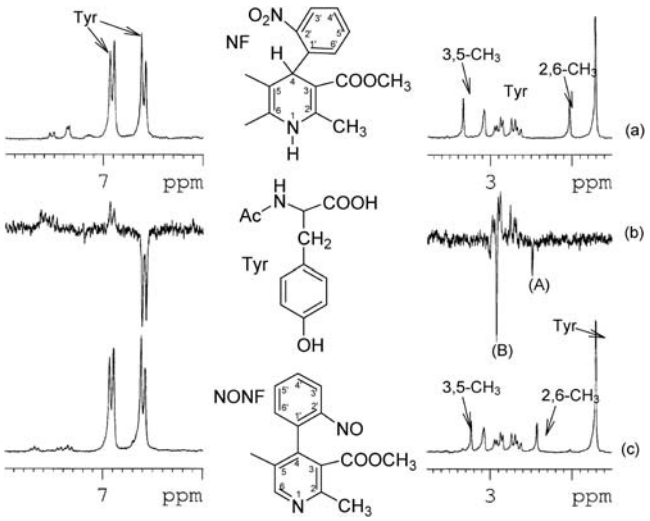


Figure 2. Aromatic and aliphatic parts of ^{1}H-NMR spectra (200 MHz) of the reaction mixture of 1 m*M* NF and 5 m*M* Tyr in D_2O–CD_3OD (5:1). (a) Initial spectrum before irradiation; (b) TR CIDNP spectrum observed during irradiation of the reaction mixture (128 laser pulses with the delay time equal to zero). Lines (A) and (B) were attributed to NONF and the corresponding pyridinium cation, respectively (see main text for details). (c) Spectrum of the reaction products after the irradiation.

method to generate the paramagnetic species and to study the detailed mechanisms of their transformations appears to be well founded. The CIDNP technique has been chosen because it is an extremely effective tool to study the mechanisms of complex chemical reactions involving paramagnetic intermediates (13,14), in particular, the reactions of analogous 1,4-dihydropyridines with various electron acceptors (10–12) and nitroaromatics with alcohols in solution (8). In addition, the application of TR CIDNP technique will allow to detect the polarizations manifested only by those diamagnetic products stemming from radical stages, thus revealing the targeted processes even in the presence of potent competing reaction pathway—the direct phototransformation of NF to NONF.

NMR spectra and the observed rates

The analysis of the NMR spectra taken after the photolysis of NF has shown that NONF is the sole reaction product both under the direct photolysis of NF (in the absence of any donor or acceptor) and during the photolysis in the presence of Tyr (Fig. 2). Chemical shifts (δ, ppm) of NF and NONF in D_2O–CD_3OD are listed below. NF: δ 2.02 (s, 2,6-CH_3), 3.33 (s, 3,5-CH_3), 5.52 (s, 4-H), 7.52 (d, 3′-H), 7.35 (t, 4′-H), 7.15 (t, 5′-H), 7.35 (d, 6′-H); NONF: δ 2.43 (s, 2,6-CH_3), 3.23 (s, 3,5-CH_3), 6.60 (d, 3′-H), 7.41 (t, 4′-H), 7.70 (t, 5′-H), 7.33 (d, 6′-H).

The relative conversion degree of the NF photodecomposition has been measured under various reaction conditions, including pure methanol, methanol–water mixtures as well as in the presence of different concentrations of Tyr. The data shown in Table 1 demonstrate that the yield of NONF is dependent on the number of the laser pulses (depth of photolysis), the reaction media and the concentration of added Tyr.

It is seen from Table 1 that the addition of water to the reaction mixture gives rise to the competition between the intramolecular phototransformations of NF and its intermolecular interactions with Tyr. One might suggest that such a suppression of the intramolecular transformation of NF to NONF is due to binding of the NO_2 group of NF to water molecules through hydrogen bonds.

Table 1. The yield of NONF on the depth of photolysis in various media

Reaction media	Yield of NONF (% initial NF)*	
	64 laser pulses	128 laser pulses
Methanol	38.5	50.0
Methanol–water (1:1)	26.7	39.0
Methanol–water (1:1) + 20 m*M* Tyr†	34.6	47.5
Methanol–water (1:1) + 50 m*M* Tyr†	36.6	49.0

*Initial concentration of NF is 5 m*M*.
†In the presence of Tyr, the conversion degree is adjusted for the light absorption by Tyr. The ratio of molar extinction coefficients of NF and Tyr at 308 nm is equal to 40.

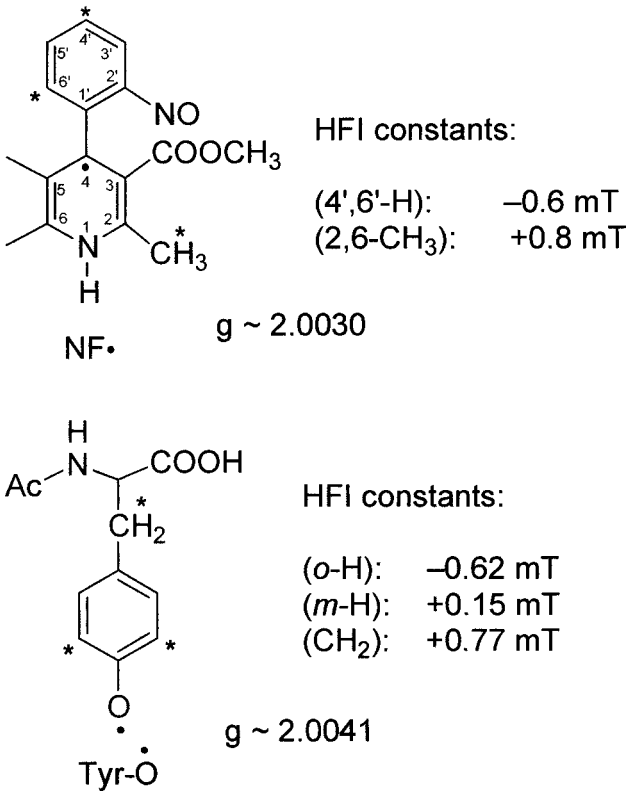
CIDNP in the photolysis in homogeneous solution

No CIDNP effects were detected during the irradiation of the individual solutions of NF or Tyr in acetonitrile or methanol solutions and in the mixture of methanol and water. Moreover, no CIDNP effects were detected during the irradiation of NF in the presence of Tyr in acetonitrile or methanol solutions. This finding is in accordance with earlier results indicating that NF is photochemically unstable and subject to quantitative intramolecular phototransformation to its nitroso form (3). We suggest that the absence of nuclear polarization in acetonitrile and methanol solutions is due to higher reaction rate of such intramolecular transformation as compared with the intermolecular reactions.

To detect the paramagnetic intermediates in the reaction of NF and Tyr, we have performed CIDNP experiments in D_2O–CD_3OD mixture. Indeed, in this case we have detected CIDNP effects of both Tyr and the product of NF transformation (NONF). The TR CIDNP spectrum (Fig. 2b) also shows polarized lines of CH_2 groups—enhanced absorption: δ 2.6–3.0 (m)—and those of aromatic protons of Tyr—enhanced absorption: δ 6.9 (d, *m*-H), emission: δ 6.7 (d, *o*-H). In the aliphatic part of TR CIDNP spectrum, two more emission lines A and B (Fig. 2b) were observed: δ 2.43 (s) and 2.85 (s), respectively. Signal A (δ 2.43) corresponds to 2,6-CH_3 protons of NONF, whereas line B (δ 2.85) presumably belongs to 2,6-CH_3 protons of the intermediate pyridinium cation of NONF. In our earlier experiments, we have observed the NMR signals of 2,6-CH_3 protons of N-substituted pyridinium cations with the identical chemical shift (δ 2.85) (11). This is a well-substantiated assumption because such a strong low field shift is more characteristic for the charged molecules (*e.g.* cations) as compared with the neutral ones. Weak absorption in the region of δ 7.45–7.65 (m) could be attributed to the 4′,6′-H of the phenyl ring of NONF and pyridinium cation; however, it is impossible to analyze these effects because of low enhancement coefficients.

The ratio of CIDNP intensities of different protons of Tyr (Fig. 2b) is an evidence that the polarized Tyr molecule was formed from the neutral Tyr radical (14,15) because this value is known to be equal to the ratio of hyperfine interaction (HFI, Scheme 2) constants of corresponding protons in the precursor radical (*ca* 4).

An additional experiment was carried out to confirm that CIDNP effects were truly formed because of the interaction of Tyr with NF



Scheme 2. Magnetic resonance parameters (radical g-factors and HFI constants) of Tyr (15) and NONF (12) neutral radicals (asterisk denotes the protons with the highest HFI constants).

but not with NONF excited molecules. Indeed, the reaction of NONF with Tyr does not result in the above-described polarization pattern shown in Fig. 2.

Note that no polarization effects were observed for the initial NF (Fig. 2b). To check for the possible back ET stage resulting in the formation of NF radical anion and to trace its transformations in solution, we have expanded the model system by adding another electron donor, TPA. In this case, TR CIDNP effects were detected for the initial NF, emission: δ 2.2 (s, H_2O), δ 7.5–7.7 (dd, 3′,5′-H), absorption: δ 2.3 (s), δ 5.6 (s, 4-H) (Fig. 3). The origin of the observed effects is clearly connected with the formation of NF radical anion and will be discussed below.

DISCUSSION

CIDNP analysis and the reaction mechanism

In the first instance, it is necessary to note that the observation of chemical polarization effects in the reaction under study is the direct evidence of the radical reaction pathway of the interaction between NF and Tyr. The following factors contribute to the process of the establishment of the detailed reaction mechanism.

CIDNP effects and their possible origin. In accordance with CIDNP theory (13), CIDNP is formed in the pair of paramagnetic particles; the sign of the observed CIDNP effect (enhanced absorption or emission) is defined by the characteristics and properties of the excited state of precursor molecules and intermediate radicals and is easily calculated by means of simple empirical rules (16). The rules take into account the multiplicity of

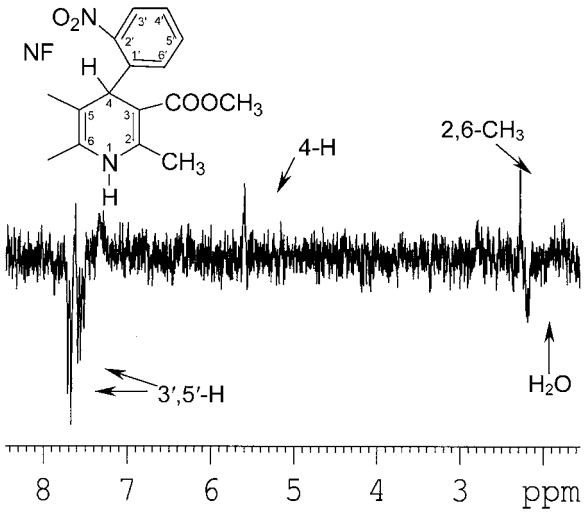
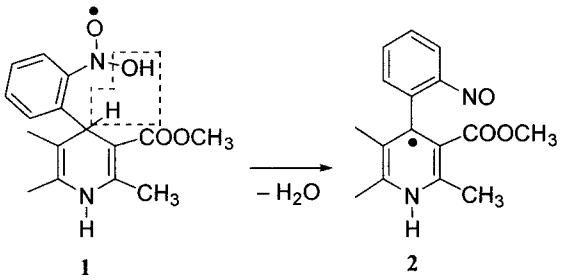


Figure 3. TR CIDNP spectrum observed during irradiation of 5 m*M* NF in the presence of 10 m*M* TPA in CD_3CN (128 laser pulses with the delay time equal to zero).

precursor radical pair, the possibility for geminate radical recombination as well as their recombination on random encounters in the bulk, the difference in the radical g-factors and the signs of the corresponding HFI constants. The polarization intensities of different proton groups in the TR CIDNP spectra are proportional to the values of HFI constants of the corresponding protons in the radical precursors of the polarized reaction products.

As it has been mentioned above, the analysis of polarization of Tyr has shown that this was formed in the neutral Tyr radical. We also suggest that the second partner of radical pair precursor NONF could also be a neutral radical. To make a conclusion about the structure of the NF free radical precursor of the reaction products, NONF and the corresponding pyridinium cation, we analyzed the polarizations of the 2,6-CH_3 protons of the reaction products, signals A and B, respectively (Fig. 2). Noticeable polarizations of the methyl protons with the intensities close to those of the aromatic ones suggest that the observed CIDNP effects of NONF are formed in the neutral radical, $NONF^{\bullet}$, with the unpaired electron localized on the dihydropyridine ring (Scheme 2). This is also corroborated by the polarization effects in the aromatic region (Fig. 2) detected for the 4′,6′-H, which is in agreement with the HFI distribution in this radical (17).

The analysis of the observed CIDNP effects under the assumption that these were generated in the radical pair of Tyr and NONF neutral radicals uses well-known magnetic resonance parameters of these intermediate species (Scheme 2). The results of the analysis demonstrate that the polarization of initial Tyr and the transformation products of NF (NONF and intermediate pyridinium cation) were formed in the triplet radical pair of these precursor radicals. Suggested ET between NF and Tyr could result in the triplet radical ion pair because the triplet excited state of NF may have sufficient energy for ET from Tyr, by analogy with nitrobenzene, where E^T(NF) is *ca* 2.5 eV (18). This also satisfies the well-known Weller–Zachariasse criterion for ET (19) because the energy of reactive excited state (E^T = 2.5 eV) exceeds the energy of radical ion pair (ΔH = 2.29 eV), which is defined as a difference between the redox potentials of donor and acceptor: $\Delta H = E^{+}_{1/2}(\text{Tyr}) - E^{-}_{1/2}(\text{NF}) = 2.29$ V, where $E^{+}_{1/2}$ (Tyr) = 0.9 V (20) and $E^{-}_{1/2}(\text{NF}) = -1.39$ V (9). Note that there is another reason



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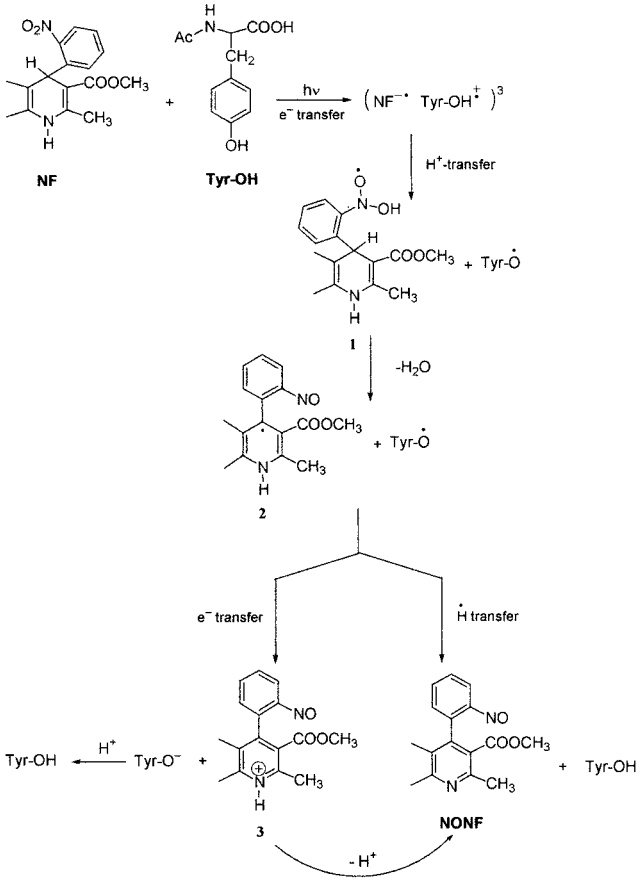
Scheme 3. Proposed mechanism of the transformation of protonated $NF^{-\bullet}$ to oxygen-centered radical of NONF.

to believe that ET could not be excluded from consideration, the possibility of the formation of the radical pair of free Tyr and NONF radicals from the radical ion pair of $TyrOH^{+\bullet}$ and $NF^{-\bullet}$ radical ions. Indeed, reference data on the application of CIDNP method to the investigations of the reactions involving Tyr show that CIDNP effects, in all cases studied, are formed in the radical pair consisting of Tyr neutral radical resulting from fast deprotonation of corresponding radical cation, although the generation of the latter has been confirmed by means of optical methods (14,21). The suggestion of the initial formation of the above-mentioned radical ion pair could also provide for the formation of the NONF free radical shown in Scheme 2.

In this context, we have attempted to detect the formation of $NF^{-\bullet}$ by means of another electron donor, TPA, with redox potential close to that of Tyr ($E^{+}_{1/2}$(TPA) = 0.92 V). Photoinduced reaction of single-electron transfer between NF and TPA has been studied in CD_3CN. Because of high molar extinction of TPA ($\varepsilon_{308} = 3 \times 10^4\ M^{-1}\ cm^{-1}$), the light is predominantly absorbed by TPA and the reaction is initiated by its exciting state. This also allows to suppress the contribution from the secondary process of direct transformation of NF to NONF. Indeed, the detected nuclear polarization effects of the initial NF (emission: δ 7.5–7.7 [dd, 3′,5′-H], absorption: δ 2.3 [s, 2,6-CH_3], δ 5.6 [s, 4-H] [Fig. 3]) with the ratio of integral intensities (the ratio of integral CIDNP intensities normalized by the number of protons of 3′-H:5′-H:2,6-CH_3:4-H equals to 4:5:0.12:1) corresponding to the HFI distribution in $NF^{-\bullet}$ radical anion (3′-H, 0.31 mT; 5′-H, 0.4 mT; 2,6-CH_3, 0.01 mT; 4-H, 0.1 mT) (9) is undoubtedly formed through reversed ET in the radical ion pair of $TPA^{+\bullet}$ and $NF^{-\bullet}$ and clearly demonstrates the presence of NF radical anion. CIDNP spectrum (Fig. 3) also shows the polarization of the water protons (emission: δ 2.2 [s, H_2O]). The appearance of polarized water protons that are present in CD_3CN in trace amounts allows to suggest further transformations of the NF radical ion. CIDNP effects of water protons have the sign opposite to that of 4-H protons of the initial NF, and this is an evidence of the fast protonation of $NF^{-\bullet}$ radical anion followed by the formation of the oxygen-centered free radical of NF and the elimination of the polarized water molecule (Scheme 3).

This mechanism is based on the reference data (3) on the transformation of nitro to nitroso group. This is also the possible path of transformation of the radical anion to the free radical in the case of the reaction with Tyr. Here, the radical could form in the cage through elimination of the water molecule from the oxygen-centered radical resulting from the proton transfer from $TyrOH^{+}$.

Reaction mechanism. Thus, the observation of CIDNP effects unambiguously points to the radical reaction of Tyr with the excited state of NF. The above experimental data and the reference



Scheme 4. General mechanism of the reaction between NF and Tyr.

data on the ET processes involving both nitroaromatics 1,4-dihydropyridines and Tyr (22–24) allow to suggest that ET is the primary stage of interaction between Tyr and NF.

The above reasoning as well as the results of CIDNP analysis could be summarized in the general scheme of the reaction between NF and Tyr, which could form the basis for future modeling of NF–receptor interaction (Scheme 4).

Pyridinium cation **3** is evidently unstable and is not observed in the equilibrium NMR spectrum after photolysis. The formation of **3** from radical **2** via the ET stage proceeds similarly to the analogous process observed in the reaction of 1,4-dihydropyridines with methyl viologen (25), where it has been shown that for reduced form of nicotinamide adenine dinucleotide and its analogs this process is characterized by diffusion-controlled rate.

Thus, the observed experimental data has allowed to propose the mechanism of transformation of NF to NONF, which is known to be one of the short-lived intermediate products of its metabolism in the living system (5,6). It is necessary to note that this is not a result of NF metabolism in liver because, as it has been mentioned above, the metabolism by liver cytochrome P-450 leads to nitropyridine, rather than to nitrosopyridine. The mechanism includes three basic steps: (1) the formation of the neutral radical **1** resulting from sequential ET from Tyr to NF, followed by proton transfer; (2) transformation of **1** to dihydropyridine neutral radical **2** after the elimination of water molecule; and (3) electron or hydrogen atom transfer regenerating the initial Tyr molecule and leading to the main reaction product NONF (Scheme 4).

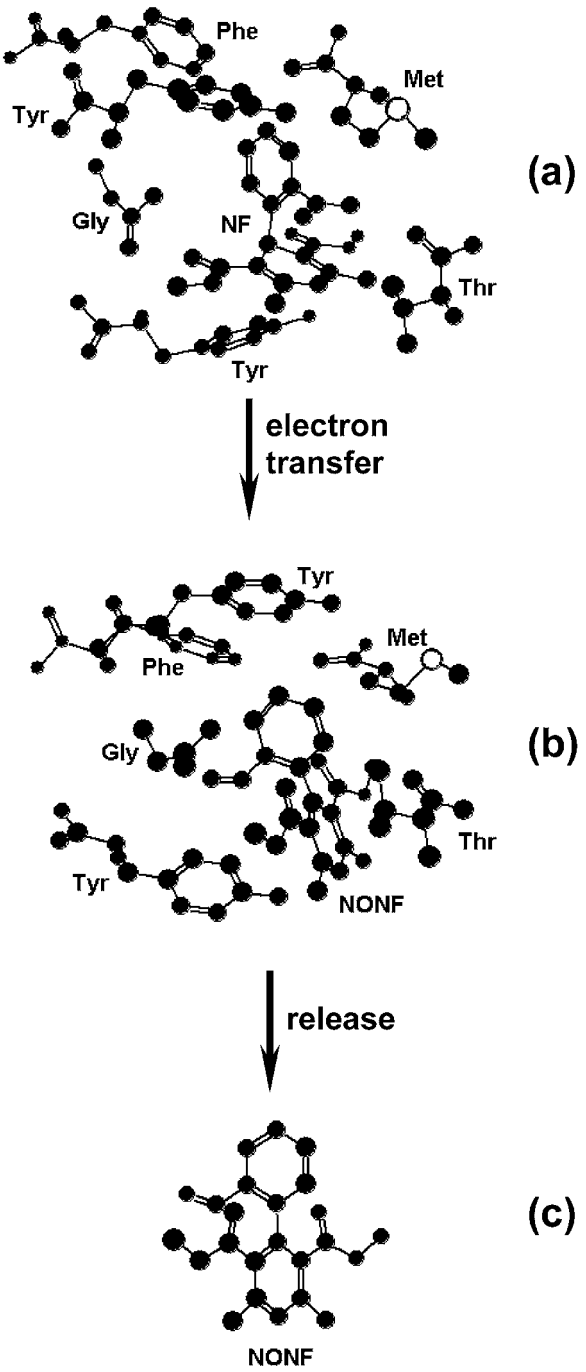


Figure 4. Schematic Chem3D Pro (Cambridge Software) presentation of the transformations of NF to NONF inside Ca^{2+} receptor active site formed by amino acid residues. Conventional notation used (Tyr, tyrosine; Phe, phenylalanine; Gly, glycine; Thr, threonine; Met, methionine). Data necessary to create these structures were partially taken from Schleifer (2); MM2 procedure has been used for energy minimization. (a) NF bound to Ca^{2+} receptor active site. (b) Structural distortions of Ca^{2+} receptor active site after the ET followed by transformation of NF to NONF. (c) NONF released.

CONCLUSIONS

The proposed mechanism of the model reaction of NF with Tyr allows to formulate the general concept of the processes that occur in the active site of the receptor during the formation of the complex with NF. As it has been mentioned above, the stability of this complex is defined by several determinants. First, it is the binding of the electron-rich dihydropyridine cycle with three amino acids of the active site; second, it includes the donor–acceptor interaction between three other amino acid residues and the nitrophenyl substituent that is believed to be accompanied by charge-transfer interaction (2,7).

In the framework of the present model the interaction between NF and Tyr is regarded to be the most significant. The reaction of single-electron oxidation of NF involving the transformation of the nitrogroup to nitroso, which has been studied in detail by means of CIDNP method, results in the aromatization of the dihydropyridine cycle into a pyridine one. This leads to the breaking of the donor–acceptor bonding of this fragment with three amino acid residues of the active site of the receptor; the spatial structure also changes. The resulting product—NONF—could not be bound to the receptor active site, and the complex dissociates, thus unlocking the receptor (Fig. 4).

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