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Mechanisms of photoinduced electron transfer reactions of lappaconitine with aromatic amino acids. Time-resolved CIDNP study

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CIDNP techniques were applied to the investigation of the elementary mechanism of photoinduced interaction between anti-arrhythmic drug lappaconitine and amino acids tyrosine and tryptophan. It has been shown that the reactions involve the formation of lappaconitine radical anion. Lappaconitine radical anion is unstable and rapidly eliminates *N*-acetyl anthranilic acid *via* protonation and ether bond cleavage. The rate constant of ether bond cleavage was estimated to be equal to 4×10^5 s^{-1}. The role of single electron transfer is discussed in the light of the model of drug–receptor interactions.

Introduction

Lappaconitine (Lap, Fig. 1) is a diterpenoid alkaloid extracted from *Aconitum Septentrionale Koelle*, which has wide pharmacological application as an anti-arrhythmic drug.[1,2] Therapeutic action of Lap is believed to be defined by the blocking of sodium channels, since it has been shown to irreversibly bind to site 2 of the Na-channel[3] with the peptide loop composed of the known sequence of amino acids.[4] What process is underlying the dissociation of the complex of the drug and site 2 cavity and the release of the Na-channel? In our recent paper, we have suggested that drug–receptor interactions could involve the chemical reactions between the molecule of the drug and amino acid residues forming the binding centers of the receptor and/or channel.[5] This approach is based upon the hypothesis that such an interaction might result in the chemical transformation of the drug leading to the loss of the binding with the receptor.

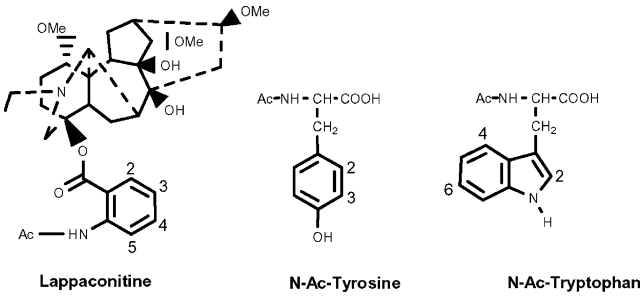

Fig. 1 Structures of lappaconitine and model amino acid residues.

The present work intends to investigate the reactions of lappaconitine with amino acids present in the peptide loop of site 2 of Na-channel. This is the second paper in a series attempting to describe the molecular mechanism of interaction of a drug with the cell receptors as a sequence of chemical reactions. The first paper was devoted to the known Ca^{2+}-antagonist, nifedipine. It has been shown that single electron transfer (SET) might be a key stage of the reaction between drug and receptor binding site.[5] Since the drug–receptor binding should be reversible, it was assumed[5] that SET results in the chemical transformation of the paramagnetic intermediate of the preparation leading to the decay of the drug–receptor complex. The radical anion of nifedipine formed after electron transfer between nifedipine and tyrosine present in the binding site of the Ca^{2+}-receptor is unstable and rapidly transforms into nitrosopyridine in the microsecond time scale. Molecular modeling shows that nitrosopyridine, in contrast to nifedipine, is incapable of binding to the receptor.

In the framework of the above hypothesis on SET, in the present work, two amino acids, tyrosine (Tyr) and tryptophan (Trp), present in the peptide loop of site 2 of the Na-channel has been chosen as the most probable candidates for the interaction with lappaconitine. Current literature lacks data on the reaction of Lap with amino acids, however, the reactions of tyrosine, tryptophan and some biologically relevant molecules involving SET stages have already been studied by means of laser pulse photolysis and CIDNP (chemically induced dynamic nuclear polarization) techniques.[6–10] In the present work, the photoinitiated CIDNP method was also used to study the interaction of Lap with the above amino acids. This is in line with the earlier elaborated approach involving application of model processes to study the fine details of the drug–receptor interactions.[5] Here, it is necessary to highlight the postulate underlying the above approach: the reactivity of active intermediates is independent of the method of their generation. In the case of single electron transfer, these are radical ions and free radicals. The present work also uses photogeneration, and the fate of the radical species is monitored by the CIDNP technique.

Hence, to solve the problem formulated above one should establish the elementary mechanisms of photoinduced interaction of Lap with tyrosine and tryptophan as well as the transformations of lappaconitine radical ions, and to discuss the possible implications of these transformations for the drug–receptor binding.

Experimental

Chemicals

Lappaconitine was extracted from the root of *Aconitum Septentrionale Koelle* in accordance with known procedure.[11] The synthesis of *N*-acetyl anthranilic acid (NAAA) is also described elsewhere.[12] Deuterated solvents D_2O and CD_3OD (Aldrich), *N*-acetyl tyrosine and *N*-acetyl tryptophan (Sigma) were used

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as supplied. The solutions (concentrations of reagents were in the range from 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 NMR spectrometer at room temperature. An 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 a DPX 200 Bruker NMR spectrometer (200 MHz ^{1}H operating frequency). In the time-resolved (TR) CIDNP experiments, to suppress the equilibrium signals a standard presaturation technique was employed involving the sequence: (i) saturating radiofrequency pulse; (ii) laser pulse; (iii) time delay; (iv) detection radiofrequency pulse; and (v) free induction decay. In the TR CIDNP experiments, a 1 μsec detecting radiofrequency pulse was used, which is approximately equivalent to a 15° pulse. Quasi steady state (QSS) CIDNP experiments were performed using the special presaturation technique: saturation–180° pulse–a train of laser pulses–evolution time–detection pulse–free induction decay. This ensures the suppression of the equilibrium NMR signals in the CIDNP spectrum, and only the signals of the products demonstrating nuclear polarization could be observed.

Analysis of reaction products

Final products of the photolysis were analyzed by means of NMR spectroscopy (DPX 200 Bruker NMR spectrometer), LC/MS with mass-selective detector Agilent 1100 Series LC/MSD, and HPLC (Millichrom A02).

Results and discussion

Reaction products

To discuss possible pathways of lappaconitine transformation in photoinduced interactions with electron donors, one should focus on the following peculiar properties of the system under study. First, in all experiments under discussion the light is adsorbed by Lap due to the high extinction coefficient ($\varepsilon \sim 10^4$ at $\lambda = 308$ nm).[13] Another determinant of the direction of the process is the bifunctionality of Lap. The amide and ester moieties of the aromatic ring (anthranilic fragment) ensure the electron acceptor properties of the molecule. On the other hand, the amine group in the aliphatic fragment of the Lap molecule possesses electron donor properties (Fig. 1). It is believed that photophysical properties of Lap are fully determined by the characteristics of the anthranilic fragment of the molecule.[13–15] The presence of two functional groups stipulating electron donor properties in the anthranilic fragment of Lap, namely amide and ester groups, allows one to suggest two possible directions of electron transfer processes. As for the ester function, it is the breaking of the ester C–O bond, while in the case of amide the electron transfer could be followed by deacetylation of the anthranilic fragment. The former process is characteristic for the photolysis of aromatic esters in the presence of electron donors,[16] and for basic hydrolysis of Lap.[17] The deacetylated form of Lap is known to be a key observable metabolite in living systems.[18,19]

NMR spectra of the reaction products after photolysis in all solvents suggests the presence of an anthranilic fragment. Chemical shifts of aromatic protons of this fragment are close to those of initial Lap. On these grounds, the pathway involving deacetylation could be rejected. Indeed, if the deacetylation takes place, the signal of the 5-CH proton of anthranilic moiety will be shifted from 8.3 ppm to 6.5 ppm. The NMR spectrum of the product in $CDCl_3$ shows the lines at ~9 ppm and 13 ppm characteristic of N-H and COOH protons. The analysis of LC/MS data on the reaction mixture also points to the formation of a product with molecular weight equal to that of *N*-acetyl anthranilic acid (NAAA). No traces of the deacetylation product were detected. To prove the hypothesis on the formation of *N*-acetyl anthranilic acid, we have synthesized NAAA and compared its NMR spectrum with that of the reaction product. NAAA was also added as a reference in GC separation of the products. The identity of the spectra allows confirmation of the formation of *N*-acetyl anthranilic acid in the reaction under study.

As for the other products resulting from the alicyclic part of Lap after elimination of NAAA, two major products have been found to have molecular weights equal to 405 and 407. One might suggest that the first product results from the loss of one proton by the alicyclic radical of Lap arising through the breaking of the ester C–O bond with the formation of a double C=C bond at the breaking site. The second product can originate through hydrogen addition to the free radical at the breaking site. The total reaction scheme will be described later in more detail, supported by the evidence for suggested pathways of lappaconitine transformation in the reaction with tyrosine.

CIDNP analysis

CIDNP effects have been detected in the photoinitiated interaction of Lap with Tyr and Trp. Polarized protons were observed for initial compounds, as well as for the product NAAA. It has been found that Lap might also undergo photocleavage *via* a free radical mechanism in the absence of amino acids. However, in this case, CIDNP effects differ substantially from the polarization pattern observed in the bimolecular reaction. The discussion of unimolecular photocleavage of Lap is beyond the scope of the present paper. The present work focuses solely on the mechanism of bimolecular interaction of Lap with amino acids. Nevertheless, it is important to note, that the reaction with amino acids proceeds with greater efficiency than photocleavage. This was clearly demonstrated by the acceleration of the disappearance of Lap, *e.g.*, in the presence of 0.07 M Tyr the rate of NAAA formation shows a two-fold increase. In the CIDNP spectrum, this is corroborated by the significant decrease of CIDNP signals observed for the photocleavage products.

It is known that the appearance of CIDNP effects unambiguously points to the participation of paramagnetic particles in the reaction. To elucidate the structures of these particles, the signs and intensities of polarized lines are compared with EPR parameters (hfi constants and *g*-factors) of suggested paramagnetic precursors of polarized products. Due to the lack of reference data on the paramagnetic derivatives of Lap, the closest analogies as well as conventional criteria were used to determine the structures of paramagnetic intermediates in the reaction of Lap with amino acids. First, it is necessary to note that CIDNP effects were detected in polar media only: acetonitrile, methanol, DMSO, and water. The appearance of chemical polarization in polar solvents favours of the electron transfer as the initial step of the reaction.

The CIDNP spectrum detected under photolysis of Lap with Trp in a water–methanol mixture (2 : 1) is shown in Fig. 2.

It is seen from Fig. 2, in the aromatic region of chemical shifts, only the protons of the anthranilic fragment of Lap and NAAA are polarized. The observed pattern allows the suggestion that the spin density distribution in paramagnetic particles resulting from Lap is close to that of radical ions and free radicals of the benzoic acid esters.[20] In this case, as well as in our experiments, the hfi constants $a_{2,4}$ and $a_{3,5}$ have opposite signs both for radical ion and free radical. Indeed, *o*- and *p*-protons of Lap demonstrate negative polarization (emission, E), while *m*-protons are polarized positively (enhanced absorption, A). Such behaviour could be supported by an example of the values of hfi constants (in G) in the radical ion of Ph-C(O)OCH$_2$CH$_3$ (4.11 (*o*), 0.78 (*m*), 7.51 (*p*)), and free radical of Ph-C(R)O (4.8 (*o*), 1.6 (*m*), 5.88 (*p*)).[20]

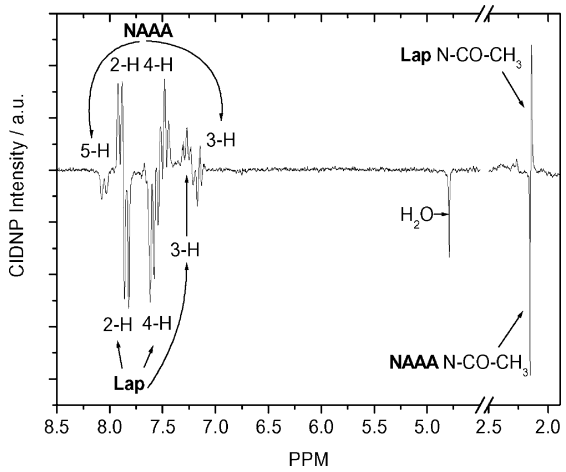
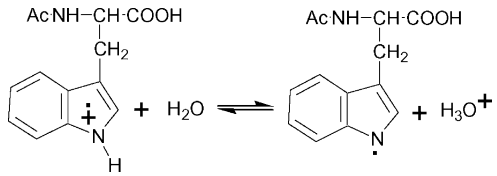


Fig. 2 QSS ^{1}H CIDNP spectrum observed under laser irradiation of Lap (3 mM) in the presence of Trp (10 mM) in a D_2O–CD_3OD mixture (2 : 1). The 5-H proton of Lap is not shown due to the overlap with other signals.

According to known empirical rules for the analysis of CIDNP effects,[21] the emission of 2,4-H of Lap is attributed to the cage product of the triplet radical ion pair (RIP) of the Lap radical anion and the Trp radical cation ($a_{2,4} < 0$; g(Lap) > g(Trp)).[22,23] The CIDNP signs of NAAA protons are opposite to those of Lap, and this points to the formation of anthranilic acid in the bulk as a result of fragmentation of the Lap radical.

Further, it is necessary to discuss the origin of polarization of the water molecules detected in the photolysis in the presence of Trp (Fig. 2). The most reasonable source of CIDNP of water protons is the deprotonation of Trp radical cations in the bulk (Scheme 1).



Scheme 1 Deprotonation of the tryptophan radical cation.

According to the reference data,[22] the deprotonation rate of the Trp radical cation is of the order of 10^6 s^{-1}. This value exceeds considerably characteristic values of spin-lattice relaxation rate in free radicals. The observation of CIDNP effects of water protons is additional evidence in favour of electron transfer, since the polarization of the NH-proton could be formed only in RIP. Neutral Trp radical lacks an NH-proton.[23]

In this experiment, the absence of CIDNP of Trp could be the result of the manifestation of the electron or proton exchange processes. These processes are capable of destroying CIDNP effects through the compensation of polarizations formed in the cage and in the bulk. Thus, to study the dynamics of polarization effects of Trp, we have measured the time dependence of CIDNP in the system under study. At the starting point, under zero time delay between laser and registration pulses, CIDNP spectrum shows only the polarizations of cage products, Lap and Trp (Fig. 3a).

The comparison of CIDNP effects detected in the present work with the reference data relating to aqueous solutions,[22] and with the computer simulated spin density distribution,[23] point to the formation of these polarizations in RIP involving a Trp radical cation. In this experiment, CIDNP analysis shows that Trp is polarized as the cage product of triplet RIP. Since in all experiments the laser irradiation is mainly absorbed by Lap, one might conclude that Lap reacts *via* electron transfer from triplet excited state.

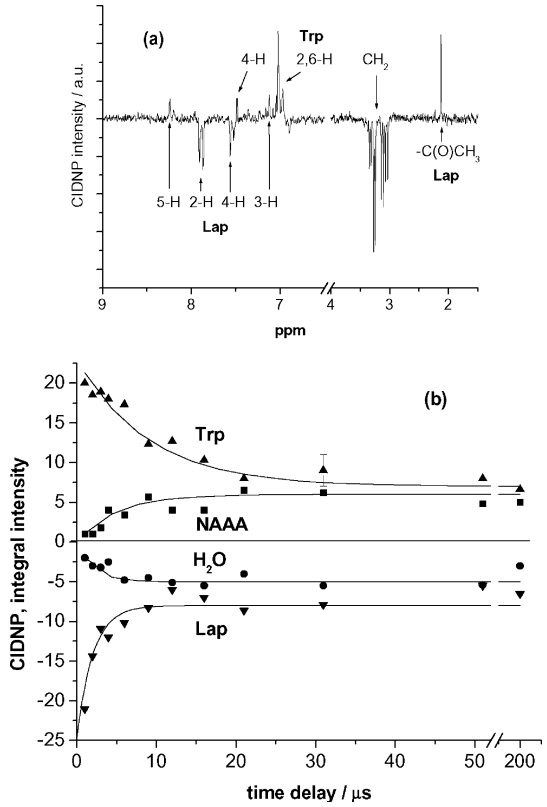


Fig. 3 (a) TR CIDNP spectrum observed under laser irradiation of Lap (3 mM) in the presence of Trp (10 mM) in CD_3OD (256 laser pulses with the delay time equal to zero between laser and registration pulses); (b) the dependences of integral CIDNP intensities of aromatic protons of lappaconitine (Lap), *N*-acetyl anthranilic acid (NAAA), Trp, and H_2O on the time delay between laser pulse and registration pulse (P1 = 1 μs).

Fig. 3b shows the time dependences of CIDNP intensities detected in this experiment. The trend of these curves confirms the conclusions made on the basis of stationary CIDNP effects: initial compounds, Lap and Trp, are polarized as the cage recombination products of RIP, while the polarizations of water and *N*-acetyl anthranilic acid are formed in the processes in the bulk. Moreover, the fast decrease over time with further plateau of CIDNP intensities of aromatic protons of Lap is evidence of fast electron exchange ($k_e \sim 1.5 \times 10^8$ M^{-1} s^{-1}) between Lap and its radical anion, which is comparable with the rate of protonation of radical anion followed by the formation of NAAA. As for tryptophan, according to its CIDNP time dependence, it participates in a much slower reaction of proton exchange ($k_p \sim 10^7$ M^{-1} s^{-1}).

CIDNP effects of Lap and NAAA have been detected also in the presence of another amino acid, tyrosine. Polarization of water molecules detected in the experiments with Trp was not observed in the case of Tyr, since the Tyr radicals lack the functional groups with significant hfi constants capable of entering the exchange processes with water.[6,24] In addition, due to fast deprotonation, CIDNP effects on Tyr molecules are consistent with the spin density distribution characteristic for free radicals, rather than the Tyr radical cation. This conclusion is based upon the ratio of CIDNP intensities of *m*- and *o*-protons of Tyr. The ratio equals 3.5 which corresponds to the ratio of hfi constants in the free Tyr radical, while in the case of radical cation, a value >7 is expected.[24] Note that all CIDNP effects of tyrosine detected earlier in redox photoinduced processes were also attributed to free Tyr radicals.[6–8,25]

To clarify the mechanism of CIDNP formation in the above reaction, we have studied the time dependence of polarization effects. Similar to the case of Trp, CIDNP effects at zero time delay were observed only for initial compounds, Lap and

Tyr (Fig. 4a). Polarized NAAA shows up after a time delay characteristic for the processes in the bulk (~10 ms). Fig. 4b shows CIDNP time dependences of the aromatic protons of Lap, NAAA and Tyr. Polarizations of CH_3-groups of Lap and NAAA have identical time dependences. CIDNP effects of Lap and Tyr show time dependences characteristic for cage recombination products. The small maximum in the region of several microseconds might arise due to the contribution from diffusion pairs formed in the bulk (F-pairs). Note that in the experiments with Trp, the contribution from F-pairs was insignificant (*cf.* Figs. 3 and 4). The further decrease of the dependences is due to the electron exchange (in the case of Lap), and proton exchange (in the case of Tyr).

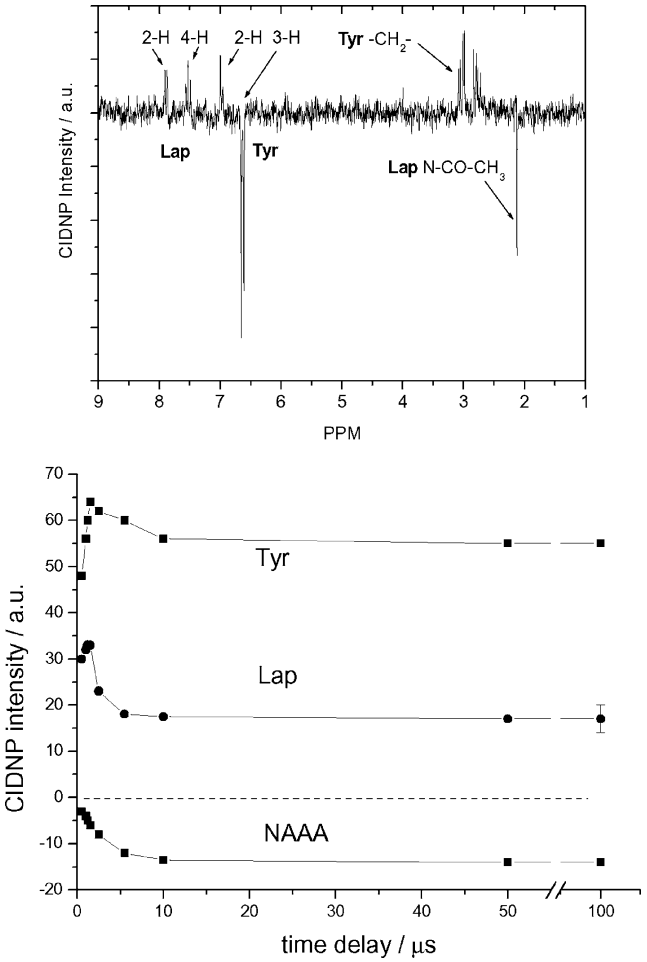
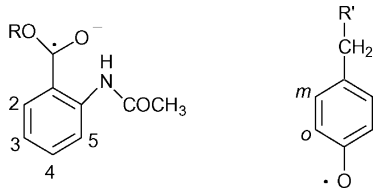


Fig. 4 (a) TR CIDNP spectrum observed under laser irradiation of Lap (3 mM) in the presence of Tyr (30 mM) in CD_3OD (256 laser pulses with the delay time equal to zero between laser and registration pulses); (b) The dependences of integral CIDNP intensities of aromatic protons of Lap, NAAA, and Tyr on the time delay between laser pulse and registration pulse (P1 = 1 μs).

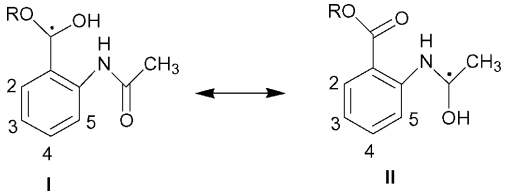
The close similarity of CIDNP effects of Lap and NAAA detected in both experiments, with Tyr and Trp, allows one to suggest that in both cases polarizations of Lap and NAAA were originated in the radical anion of lappaconitine. Again, CIDNP effects in both cases appear only in polar solvents. All these experimental results point to the electron transfer as the first stage of the interaction of Lap with amino acids. However, as has mentioned above, the tyrosine radical forms the pair in its deprotonated form. Thus, one might suggest that the tyrosine radical is formed through the deprotonated form of Tyr (anion) *via* the following reaction:

$$\text{Tyr–O}^- + \text{Lap} \rightarrow \text{Tyr–O}^{\bullet} + \text{Lap}^{\bullet -}$$

Thus, CIDNP in this system is generated in the pair of Lap radical anion and free Tyr radical:



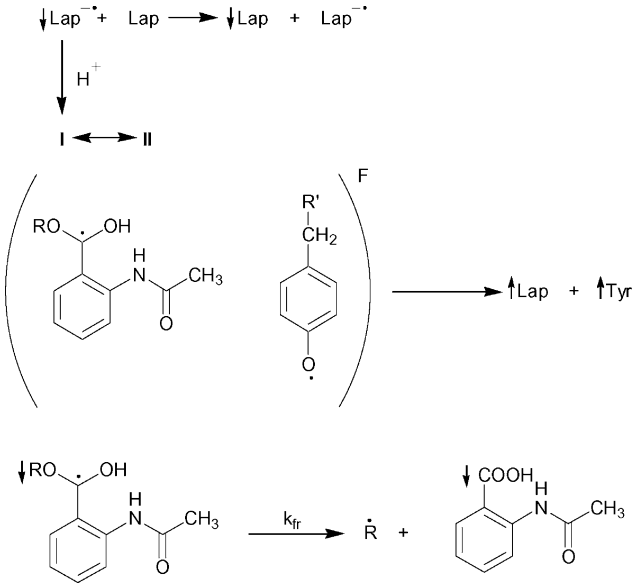
The pattern of CIDNP time dependence of Lap (fast ending to plateau) also points to the acceleration of the protonation of Lap radical anion in the presence of Tyr as compared to Trp. This is in agreement with pK values of Trp (4.7)[22] and Tyr (2.2).[25] However, while invoking only one RIP, it is impossible to describe all observed CIDNP effects. One also should not exclude the possibility of CIDNP formation in F-pairs in the bulk. In particular, the use of this pair provides no explanation for the polarizations of CH_3-protons of Lap and NAAA, since the spin density on CH_3-groups in the radical anion of Lap is too low to ensure the formation of noticeable polarizations. One might suggest that protonation of radical anion affects both carbonyl groups—ester and amide functions—with fast equilibrium between these two structures (Scheme 2).



Scheme 2 The two protonated structures of free Lap radicals.

This hypothesis is supported by the disappearance of polarization of CH_3-groups with increase of pH of the solution. This is due to the decrease of the protonated form of the radical anion of Lap.

Scheme 3 summarizes the processes in the bulk involving the Lap radical anion and free radicals.



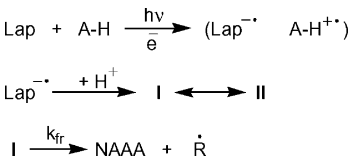
Scheme 3 Reactions of radical ions and free radicals of Lap in the bulk.

In this scheme, the polarized products formed in the cage and in the bulk are marked by the symbols ↑ and ↓. Using CIDNP dependence of NAAA formed through the fragmentation of the Lap free radical, one can estimate the rate constant of this reaction (k_{fr}). The estimation was based on the assumption that CIDNP kinetics is determined by the contribution of three different processes: (1) fragmentation of radical **I** followed by the formation of NAAA; (2) nuclear relaxation in the radical; and

(3) the decay of polarized radicals in other processes. However, taking into account that the CIDNP intensity of NAAA almost equals that of Lap, one might conclude that the fragmentation rate exceeds the rates of all the other processes, and one-exponential approximation could be used to estimate k_{fr}. The value of the fragmentation rate constant calculated under this assumption approximately equals 4×10^5 s^{-1}.

Reaction mechanism

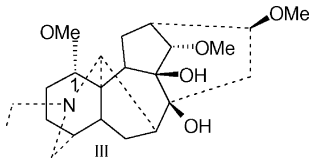
The analysis of CIDNP effects and the reaction products allows us to draw a conclusion that the mechanism of photoinitiated interaction of Lap with amino acids is similar to that described in the literature for the photolysis of esters in the presence of electron donors. It is known that the characteristic feature of this process is the breaking of ester C(O)O–R bond with the formation of corresponding acid.[16] Scheme 4 shows the suggested mechanism of phototransformation of Lap in the presence of amino acid. The detailed distinctive peculiarities of the reaction mechanisms for Tyr and Trp were described above, and these are related to the differences in the structures of paramagnetic intermediates derived from amino acids. Since the same products are formed in both reactions, with Tyr and Trp, one might suggest that Lap phototransformation in the presence in both amino acids obeys the same mechanisms.



Scheme 4 Photoinitiated reaction of Lap with amino acids.

Thus, the above reasoning allows us to propose a three-stage mechanism of photoinitiated interaction of Lap with amino acids. The ***first stage*** involves the electron transfer from the amino acid to the anthranilic fragment of Lap. The ***second stage*** is the protonation of Lap radical anion in the bulk followed by the formation of free Lap radicals, I and II, in fast equilibrium. And the ***third stage*** is the fragmentation of the free radical I resulting in the formation of *N*-acetyl anthranilic acid and the alicyclic residual of lappaconitine, R (Scheme 4).

The structures of the radicals I and II are shown in Scheme 2. In Scheme 4, A-H denotes the amino acid. As follows from product analysis, further transformations of radical R involves hydrogen abstraction from the solvent or the another radical resulting in the formation of final product R-H: 1a,14a,16b-trimethoxy-8,9-*N*-ethyl-18-noraconan (**III**).



III

Conclusion and possible biological relevance

It has been demonstrated that the electron transfer from amino acids to lappaconitine results in the formation of the lappaconitine radical anion which is extremely unstable. This radical undergoes fast decay with the formation of *N*-acetyl anthranilic acid and 1a,14a,16b-trimethoxy-8,9-*N*-ethyl-18-noraconan. Note that the products of this transformation of Lap do not demonstrate pharmacological activity. One might suggest the possibility of electron transfer between Lap and amino acid residues in the drug–receptor complex inside the Na-channel. As a result, the cleavage of the ester bond leads to the loss of drug–receptor binding. In the previous paper[5] devoted to the interaction of nifedipine with Ca^{2+} receptor, the dissociation of drug–receptor complex was also associated with the electron transfer between the amino acid tyrosine and the drug with the formation of the unstable nifedipine radical anion. It was demonstrated that the final product of that radical transformation is incapable of binding to the Ca^{2+} receptor. Thus, these two examples support the hypothesis of the possible role of the simplest chemical interaction, electron transfer, in the processes of drug–receptor binding. These facts speak in favour of the suggestion that the reversibility of drug–receptor binding might be stipulated by chemical transformation of the drugs in their complex with receptor.

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References

1 F. N. Dzhakhangirov, M. N. Sultankhodzhaev, B. Tashkhodzhaev and B. T. Salimov, *Chem. Nat. Compd. (Engl. Transl.)*, 1997, **33**, 190.
2 J. F. Heubach and A. Schuele, *Planta Med.*, 1998, **64**, 22.
3 S. N. Wright, *Mol. Pharmacol.*, 2001, **59**, 183.
4 M. E. Gellens, A. L. George Jr., L. Chen, M. Chahine, R. Horn, R. L. Barchi and R. G. Kallen, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 554.
5 N. E. Polyakov, M. B. Taraban and T. V. Leshina, *Photochem. Photobiol.*, 2004, **80**, 565.
6 Y. P. Tsentalovich, J. J. Lopez, P. J. Hore and R. Z. Sagdeev, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 2002, **58**, 2043.
7 R. Kaptein, K. Dijkstra and K. Nicolay, *Nature*, 1978, **274**, 293.
8 R. Kaptein, in: *NMR Spectroscopy in Molecular Biology*, B. Pullman, Ed., Reidel, Dordrecht, 1978, p. 211.
9 P. J. Hore and R. W. Broadhurst, *Prog. NMR Spectrosc.*, 1993, **25**, 345.
10 P. J. Hore, S. L. Winder, C. H. Roberts and C. M. Dobson, *J. Am. Chem. Soc.*, 1997, **119**, 5049.
11 S. A. Osadchii, N. A. Pankrushina, M. M. Shakirov, E. E. Shults and G. A. Tolstikov, *Russ. Chem. Bull.*, 2000, **3**, 557.
12 A. Kaufmann, *Ber.*, 1909, **42**, 3480.
13 S. S. Ostakhov, E. M. Tsyrlina, S. G. Yunusova, M. S. Yunusov and V. P. Kazakov, *Russ. Chem. Bull.*, 1977, **46**, 705.
14 A. Beeby and A. E. Jones, *J. Photochem. Photobiol. A: Chem.*, 2001, **64**, 109.
15 S. A. Ross and S. W. Pelletier, *Tetrahedron*, 1992, **48**, 1183.
16 I. Saito, H. Ikehira, R. Kasatani, M. Watanabe and T. Matsuura, *J. Am. Chem. Soc.*, 1986, **108**, 3115.
17 S. W. Peletier, N. V. Mody and R. S. Sawhney, *Can. J. Chem.*, 1979, **57**, 1652.
18 F. M. Xie, H. C. Wang, J. H. Li, H. L. Shu, J. R. Jiang, J. P. Chang and Y. Y. Hsieh, *Biomed. Chromatogr.*, 1990, **4**, 43.
19 F. M. Xie, H. C. Wang, H. L. Shu, J. H. Li, J. R. Jiang, J. P. Chang and Y. Y. Hsieh, *J. Chromatogr. B: Biomed. Sci. Appl.*, 1990, **525**, 109.
20 *Landolt-Börnstein New Series, Numerical Data and Functional Relationships in Science and Technology, Magnetic Properties of Free Radicals, V. II/9, part b*, H. Fischer and K.-H. Hellwege, ed., Springer-Verlag, Berlin, 1977.
21 R. Kaptein, *Chem. Commun.*, 1971, 732.
22 Y. P. Tsentalovich, O. B. Morozova, A. V. Yurkovskaya and P. J. Hore, *J. Phys. Chem. A*, 1999, **103**, 5362.
23 S. E. Walden and R. A. Wheeler, *J. Phys. Chem.*, 1996, **100**, 1530.
24 M. Tomkiewicz, R. D. Mc Alpine and M. Cocivera, *Can. J. Chem.*, 1972, **50**, 3849.
25 Yu. P. Tsentalovich and O. B. Morozova, *J. Photochem. Photobiol. A: Chem.*, 2000, **131**, 33.