Photosensitization and photoprotection properties of nicotinic acid derivatives

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The photosensitizing action of nicotinic acid, nicotinamide, and nicotinehydroxymethylamide on the photooxidation of glycyltryptophan (Gly-Trp) in an aqueous solution using UV light (240—410 nm) was found. The photooxidation was monitored by measuring chemiluminescence (CL) resulting from the decay of one of the oxidation products, dioxethane. The photosensitizing action decreases in the following sequence: nicotinamide > nicotinehydroxymethylamide > nicotinic acid. The addition of benzoquinone (0.01 mmol L^{-1}) results in a substantial decrease in the yield of sensitized CL, which indicates that the superoxide radical anion participates in the photooxidation.

Key words: nicotinic acid; nicotinamide; nicotinehydroxymethylamide; reduced nicotinamide adenine dinucleotide; photooxidation, photosensitization, inhibition; chemiluminescence.

Current interest in studying the photosensitization and photoprotection properties of drugs, vitamins, and other substances is due to a recent increase in the role of UV radiation as a damaging factor in the environment. This is associated with both changes in the boundary of optical transmission of the atmosphere due to a decrease in the ozone content and the broad use of sources of UV radiation in medicine and industry.[1—4]

Noxious results of UV irradiation increase when an organism contains photosensitizers, which include several vitamins and drugs.[4—7] At the same time, drugs can perform photoprotection functions acting, for example, as acceptors of free radicals formed by irradiation.[8] The present work is devoted to the study of the photosensitization and photoprotection properties of nicotinic acid (NA), nicotinamide (NAm), nicotinehydroxymethylamide (NHM), and reduced nicotinamide adenine dinucleotide (NAD-H).

Nicotinic acid (vitamin PP) and its derivatives are components of coenzymes and are used as vasodilating medicines as well as for prophylaxis and medical treatment of pellagra and diseases of the gastroenteric tract, the liver, and other organs.[9]

Experimental

Glycyltryptophan (Gly-Trp) (Reanal), NA and NAm (NPO Vitamin), NHM (pharmaceutical drug), and NAD-H (Boehringer) were used.

The photosensitization and photoprotection properties of nicotinic acid derivatives (ND) were studied by their effect on the chemiluminescence (CL) that accompanies the photooxidation of Gly-Trp peptide.

Study of the photosensitizing action of ND. Solutions of Gly-Trp (with or without additions of ND) in a 0.01 *M* phosphate buffer (pH 7.4) were irradiated at room temperature using the UV light from a DRK-120 high-pressure mercury lamp through BS-4 and UFS-1 glass filters or without light filters. The thickness of the irradiated layer of the solution was 0.5 cm, and the duration of the irradiation was 60 s. The solution was continuously stirred with a magnetic stirrer during irradiation. The CL signal was measured 15 s after the irradiation was stopped to exclude the short-lived CL of the buffer solution. In order to monitor the signal, the irradiated solution was pumped into the cell of a high-sensitive photometric unit.[10] When experiments were performed in an atmosphere of O_2, oxygen was passed through the solution for 3 min. Irradiation was started 1 min after the oxygen flow was stopped, *i.e.*, after the equilibrium concentration of oxygen was established in the solution.

Study of the photoprotecting action of ND. Irradiation was carried out using light with the following wavelengths: (1) λ = 436 nm in the presence of the sensitizer riboflavin ($2 \cdot 10^{-6}$ mol L^{-1}), which generates the superoxide anion ($O_2^{\cdot -}$); (2) λ = 546 nm in the presence of the dye Rose Bengal ($1 \cdot 10^{-5}$ mol L^{-1}), which produces singlet oxygen ($^1O_2$); and (3) λ > 290 nm in the presence of hydrogen peroxide (0.3 mol L^{-1}), which gives hydroxyl and superoxide radicals as well as free radicals of additives. The concentration of the initial solution of H_2O_2 was determined by the densimetric method.

Results and Discussion

Photosensitizing action of nicotinic acid derivatives

All studied ND absorb light in the UV spectral region (λ_{max} = 260 nm, ε = 2900 L mol^{-1} cm^{-1}).[11] In

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addition, NAD-H exhibits an additional absorption band in the region of 330—340 nm.[12] The chemiluminescence method used for testing the photosensitization activity is based on the fact that the yield of CL during UV irradiation of solutions of tryptophan-containing peptides increases in in the presence of photosensitizers.[10] A BS-4 light filter transmitting light with $\lambda > 290$ nm is usually used for testing medicines for photosensibilization activity. However, in the case of ND, which have no considerable absorption in this region, the sensitization effect is weak. Therefore, all main experiments were carried out using an UFS-1 light filter, which transmits in the 240—420 nm region. Taking into account the absorption spectrum of ND and the emission spectrum of a medium-pressure mercury lamp, the line at $\lambda = 254$ nm should be the most efficient one.

Under these conditions, NA, NHM, and NAm enhanced and NAD-H decreased the intensity of CL of glycyltryptophan. Nicotinamide was the most efficient sensitizer, and NA was the weakest one. The dependences of the relative yield of CL $y = I/I_0$ (I_0 is the intensity of CL in the absence of ND, I is the intensity of Cl in the presence of additives) on the content of ND in the irradiated solution reach a maximum at the concentration of the sensitizer of an order of 1 mmol L^{-1} (Fig. 1, *a*). The optical density of the irradiated solutions at these concentrations is greater than unity; therefore, it can be assumed that the occurrence of a plateau is related to the screening effect. The dependences presented in Fig. 1, *a* can be linearized in the coordinates $\log [1 - (y - 1)/y_{max}] - C_{ND}$, where y_{max} is the limiting value of amplification and C_{ND} is the concentration of the corresponding derivative (Fig. 1, *b*). The slope of the line (1300 L mol^{-1}) coincides with the value of the product εl for ND at $\lambda = 254$ nm. This linear dependence corresponds to the following equation:

$$y = 1 + y_{max}(1 - 10^{-D}), \qquad (1)$$

where $D = \varepsilon l C_{ND}$. The y values calculated from this formula agree well with the experiment up to the concentrations of ND of ~1 mmol L^{-1} (see Fig. 1, *a*). The decrease in the yield of CL at high concentrations is probably associated with intermolecular interactions.

The dependences of the intensity of CL (in relative units) on the concentration of Gly-Trp (Fig. 2) are satisfactorily described by the equation

$$I = I_\infty C_{Gly\text{-}Trp}/(C_{Gly\text{-}Trp} + a), \qquad (2)$$

where I_∞ is the limiting intensity of CL and a is the dimensionality parameter of the concentration.

Similar dependences have been observed previously for X-ray chemiluminescence and photosensitized CL.[10] They indicate that Gly-Trp is involved in competitive processes leading to both an increase and decrease in the yield of CL.

Experiments on the effect of oxygen and benzoquinone (acceptor of superoxide radicals) on the effi-

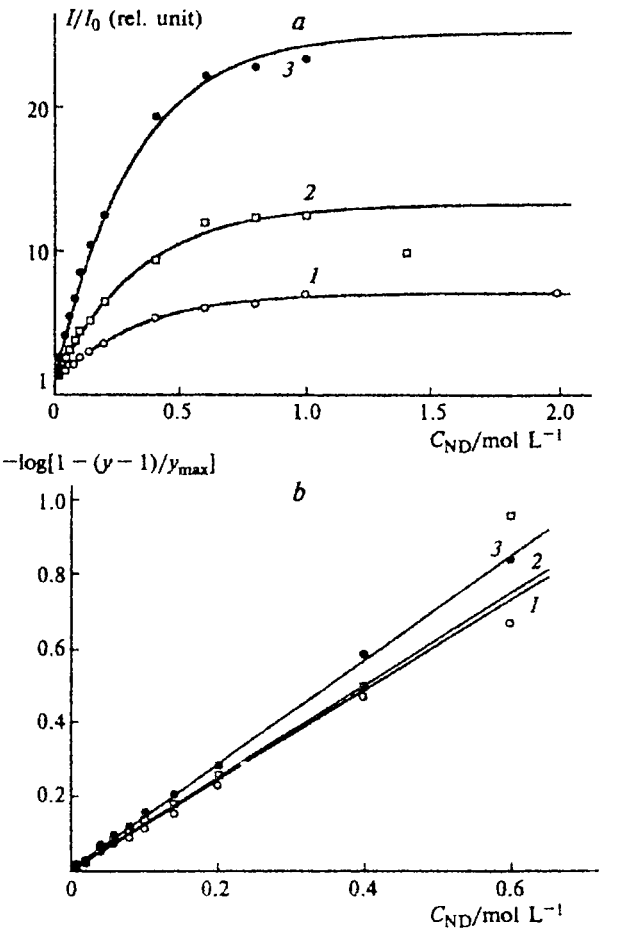


Fig. 1. *a*. Dependence of the relative yield of CL ($y = I/I_0$) on the concentration of ND: *1*, NA; *2*, NHM; *3*, NAm; $C_{Gly\text{-}Trp} = 0.1$ mmol L^{-1}. Points indicate experimental data, solid lines indicate calculation by Eq. (1).
b. Linear anamorphoses of the dependences presented in Fig. 1, *a* (for designations, see Fig. 1, *a*).

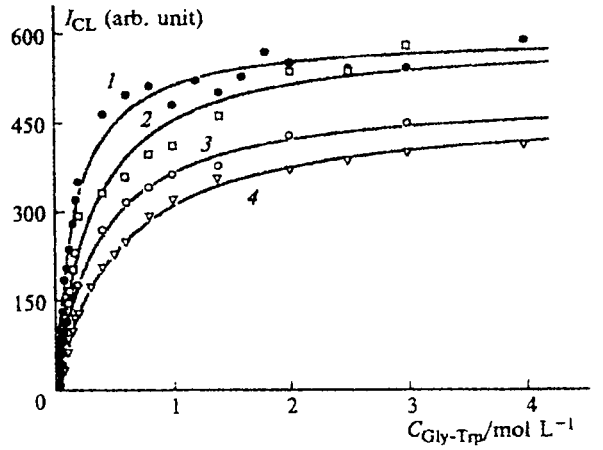


Fig. 2. Dependence of the intensity of CL on the concentration of Gly-Trp in the presence of ND: *1*, NA (0.04 mmol L^{-1}); *2*, NHM (0.02 mmol L^{-1}); *3*, NAm (0.02 mmol L^{-1}); *4*, in the absence of additives. Points indicate experimental data, solid lines indicate calculation by Eq. (2).

Table 1. Effect of oxygen and benzoquinone on the yield of CL at concentrations of Gly-Trp from 0.02 to 0.40 mmol L^{-1}

Compound	I_O/I_A [a]					I_{BQ}/I_0 [b]
	0.02 [c]	0.05 [c]	0.10 [c]	0.20 [c]	0.40 [c]	
NA	1.00	1.00	1.00	0.98	1.05	0.16
NHM	0.98	1.00	0.95	0.95	1.02	0.10
NAm	0.74	0.79	0.87	0.82	1.00	0.09

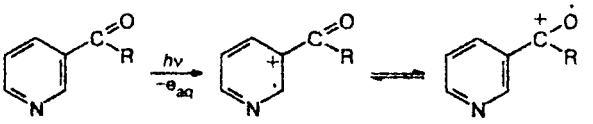
[a] I_A and I_O are the intensities of CL at the concentrations of oxygen in an aqueous solution equilibrium with air (0.28 mmol L^{-1} at 20 °C) and equilibrium with oxygen at atmospheric pressure (1.3 mmol L^{-1}), respectively.
[b] I_{BQ} and I_0 are the intensities of CL in the presence of benzoquinone ($1 \cdot 10^{-5}$ mol L^{-1}) and in the absence of benzoquinone, $C_{Gly\text{-}Trp} = 0.1$ mmol L^{-1}.
[c] $C_{Gly\text{-}Trp}$/mmol L^{-1}.

ciency of sensibilization were carried out in order to reveal the mechanism of the photosensitizing action of ND.

Experiments with different oxygen concentrations make it possible to elucidate the role of $^1O_2$ and triplet states of the sensitizer in the appearance of CL.[13] For this purpose, the ratio of the intensities of CL was determined when the experiment was carried out in an atmosphere of oxygen and when it was carried out in air. It turned out that oxygen exerts no effect on the sensibilization of NA and NHM and decreases the yield of CL in the presence of NAm (Table 1). Benzoquinone added in a concentration of 0.01 mmol L^{-1} decreases substantially the yield of CL sensitized by ND (see Table 1). These results show that the photosensitizing action of ND is not related to the formation of $^1O_2$. If this were not the case, we would observe an enhancement of CL as the concentration of oxygen increases; in addition, small concentrations of benzoquinone do not affect reactions involving $^1O_2$.

The effect of the addition of benzoquinone allows us to assert that the reactions of the radical anion $O_2^{\cdot -}$ play the main role in the appearance of CL. The mechanism of its formation is not quite clear. The superoxide radical anion can be generated in the reactions of triplet states of ND by analogy with other sensitizers, for example, riboflavin.[13] An increase in the oxygen content at small concentrations of Gly-Trp should result in a strong decrease in the yield of CL (3—4-fold) due to the efficient quenching of the triplet states by oxygen. In the experiments with ND, a decrease in the intensity of CL (by 25%) was observed only for NAm, which confirms the participation of NAm^T in the formation of $O_2^{\cdot -}$. For other ND, triplet states either do not appear or have very short lifetimes.

Another possible route for the appearance of $O_2^{\cdot -}$ is the photoionization of ND followed by the interaction of a hydrated electron with oxygen. The following ionization scheme can be suggested.



The degree of conversion of this process depends on the stability of the radical cation formed. The most stable radical cation should be formed in the case of NAm due to the positive mesomeric ($+M$) effect of the amino group. The negative induction ($-I$) effect of the hydroxyl group results in a decrease in the stability of the radical cations of NA and NHM.

This hypothesis explains the efficiency effect of the drugs as sensitizers. However, it is impossible finally to conclude which process is the primary stage of sensitization: photoionization or the formation of triplet states. Neither concept contradicts the published and experimental data.

Photoprotecting action of nicotinic acid derivatives

When solutions of Gly-Trp are irradiated with UV light through an UFS-1 light filter in the presence of NAD-H, a decrease in the intensity of CL is observed, which is evidence for the photoprotecting action of NAD-H. The intensity of CL decreases 2.5-fold at $C_{NAD\text{-}H} = 0.02$ mmol L^{-1} and decreases 5.8-fold at $C_{NAD\text{-}H} = 0.1$ mmol L^{-1}. Under the experimental conditions, the optical densities of NAD-H in the solution do not exceed 0.05 and 0.25, respectively. Therefore, this effect cannot be explained by screening and is probably related to scavanging of the free radicals that participate in the appearance of CL.

Experiments using H_2O_2 as the photoinitiator of CL of glycyltryptophan (irradiation with light with $\lambda > 290$ nm) and the photosensitizers riboflavin ($\lambda = 436$ nm) and Rose Bengal ($\lambda = 546$ nm) were carried out to study the photoprotecting action of ND in more detail. When hydrogen peroxide was used as the initiator, NAD-H in a concentration of 0.04 mmol L^{-1} halved the intensity of CL. Of the other ND studied, only NHM exhibited inhibition activity: a decrease in the intensity of CL by 30% was observed at $C_{NHM} = 4$ mmol L^{-1}. However, this effect is two orders of magnitude weaker than that in the case of NAD-H. In this system, the hydroxyl radical $^{\cdot}OH$, the radical anion $O_2^{\cdot -}$, and the peroxide radical of glycyltryptophan $RO_2^{\cdot}$ are the main active species participating in the appearance of CL. Compounds reacting with one or several these radicals cause a decrease in the yield of CL.

When riboflavin is used as the photosensitizer, NAD-H and NHM decrease the yield of CL. The experimental data are linearized in the coordinates $I_0/I - C_{ND}$. For NAD-H, the concentration of ND

necessary for 50% inhibition is equal to 0.04 mmol L^{-1}, and in the case of NHM, it is 0.6 mmol L^{-1}. In this system, the yield of CL can decrease in the presence of acceptors of $O_2^{\cdot -}$ and $RO_2^{\cdot}$ radicals and quenchers of the triplet states.

In the system with Rose Bengal used for generation of $^1O_2$, the concentration of NAD-H necessary for 50% inhibition was 0.6 mmol L^{-1}. The rate constant of quenching of the triplet states of Rose Bengal under the action of NAD-H is equal to $8.5 \cdot 10^8$ L mol^{-1} s^{-1} (see Ref. 14). The calculation using the published data on the lifetime of the triplet states of Rose Bengal[1] (68 μs) and the rate constant of their quenching by oxygen ($1.5 \cdot 10^9$ L mol^{-1} s^{-1})[15] showed that the observed decrease in the intensity is caused almost completely by quenching of the triplet states.

Thus, NA, NAm, and NHM are sensitizers of the photooxidation of the Gly-Trp peptide during irradiation with light in the λ = 240—420 nm range. The efficiency of sensitization increases in the order NA < NHM < NAm and correlates with the structure of the compounds studied. In addition, NHM and NAD-H exhibit photoprotective action related to quenching of the triplet states and reactions with the superoxide and/or peroxide radicals of Gly-Trp.

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