Formation of S-Nitrosothiols from Regiospecific Reaction of Thiols with N-Nitrosotryptophan Derivatives*

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S-nitrosothiols transport nitric oxide in vivo, and so-called transnitrosation reactions (i.e. the transfer of the nitroso function from nitrosotroopan to thiolate) are believed to be involved in this process. In the present study, we examined the N-nitrosotryptophan derivative-dependent nitrosation of thiols, a hitherto ignored possibility for the formation of S-nitrosothiols. The corresponding products were identified by 15N-NMR spectroscopy. The fact that the reaction proceeded under hypoxic conditions as well as in non-aqueous solution strongly indicated the occurrence of a transnitrosation reaction. Interestingly, S-nitrosothiols could only very slowly transnitrosate N-terminal-blocked tryptophan derivatives like melatonin in non-aqueous solution but did not induce such a reaction in water. The indole moiety of the N-nitrosotryptophan derivatives was fully restituted during the reaction with thiols, as demonstrated by both capillary zone electrophoresis and fluorescence spectroscopy. A determination of the Arrhenius parameters demonstrated that the corresponding rate constants were comparable with the ones known for the transfer of the nitroso function from nitrosothiol to thiolate. Thus, N-nitrosotryptophan-dependent nitrosation of thiols may occur in vivo and might offer the possibility of developing a new class of vasodilative drugs.

Nitric oxide is an important mediator in various physiological processes such as vasodilatation (1), neurotransmission (2), and immune reactions. NO is a short-lived intermediate in blood because it is rapidly trapped by the heme iron of hemoglobin and, therefore, one must conclude that nitric oxide uses a transporter to prolong its life-time (3, 4). Recently, endogenous S-nitrosothiols have been suggested to be potential ‘NO carriers (5, 6).

Although many studies were performed to investigate the physiological impact of S-nitrosothiols, the formation of RSNOs1 in vivo is not clearly understood. Two nitrosating entities have been established as being responsible for the nitrosation of thiols/thiolates in vitro at physiological pH. The first nitrosating agent is peroxynitrite (authentic material and in situ generated from NO/O2-releasing systems), which nitrosates thiols with a yield of 0.06–0.8%, but there is disagreement in the literature about the underlying mechanisms (7, 8).

The second nitrosating compound is N2O3, which is formed, as shown in Equations 1 and 2,

\[ 2 \text{NO} + \text{O}_2 \rightarrow 2 \text{NO}_2 \quad k_1 = 2.9 \times 10^9 \text{M}^{-2} \text{s}^{-1} \] (Eq. 1)
\[ \cdot \text{NO} + \cdot \text{NO}_2 = \text{N}_2\text{O}_3 \quad k_2 = 1.1 \times 10^9 \text{M}^{-2} \text{s}^{-1} \] (Eq. 2)

duringautoxidation of ‘NO (9, 10). It is supposed that nitrosation of the thiols, as illustrated in Equation 3,

\[ \text{RSH} + \text{N}_2\text{O}_3 \rightarrow \text{RSNO} + \text{H}^+ + \text{NO}_2^- \quad k_3 = 6.4 \times 10^6 \text{M}^{-1} \text{s}^{-1} \] (Eq. 3)

proceeds by a N2O3-dependent electrophilic substitution reaction (9). Additionally, S-nitrosothiols may be formed, as demonstrated in Equation 4,

\[ \text{RS}^- + \text{N}_2\text{O}_3 \rightarrow \text{RSNO} + \text{NO}_2^- \quad k_4 = 1.8 \times 10^8 \text{M}^{-1} \text{s}^{-1} \] (Eq. 4)

from the highly effective reaction of thiolate anions with N2O3 (8). It has also been demonstrated recently, as shown in Equation 5,

\[ \text{H}^+ + \text{NO}_2^- \quad k_5 = 4.4 \times 10^7 \text{M}^{-1} \text{s}^{-1} \] (Eq. 5)

that N-terminal-blocked tryptophan derivatives can be effectively nitrosated at the nitrogen atom of the indole ring by N2O3 at physiological pH (10). On account of theoretical considerations, N2O3 should be able to nitrosate tryptophan and cysteine with comparable efficiency. In contradiction to this conclusion, the formation of N-nitrosotryptophan in proteins has not yet been detected by mass spectrometry methods in previous studies. One possible explanation for this failure may be that low molecular weight metabolites such as ascorbic acid denitrosate N-nitrosotryptophan derivatives under the release

* The abbreviations used are: RSNO, S-nitrosothiol; NAP, N-acetylpenicillamine; CM-BSA, carboxymethyl bovine serum albumin; GS, thiolate ion; GSNO, S-nitrosothiol; NAT, N-acetyltryptophan; NANT, N-acetyl-N-nitrosotryptophan; SNAP, S-nitroso-N-acetylpenicillamine.

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of NO and the restitution of the indole ring. Moreover, N-nitrosotryptophan derivatives, but not S-nitrosothiols, are hydrolyzed to nitrile and tryptophan derivatives, thus rendering the half-life to 140 min (10, 11).

In the present study we identified for the first time a further N-nitrosotryptophan-depleting reaction, i.e. the transfer of the nitroso function from N-nitrosotryptophan derivatives to thiols. This putatively important reaction is fully characterized by using UV-visible absorption, fluorescence spectroscopy, capillary zone electrophoresis, and 15N-NMR-spectrometry. Interestingly, such a transnitrosation reaction proceeds in an aqueous solution with an efficacy comparable with that of the transfer of the nitroso function between S-nitrosothiols and thiols.

**MATERIALS AND METHODS**

**Chemicals—**N-Acetyltryptophan, cysteine, glutathione, and N-acetyl-penicillamine (NAP) were obtained from Sigma. Na15NO2 labeled with 99.3% 15N was purchased from Aldrich/Isotec Inc. All other chemicals of the highest purity were commercially available.

**Experimental Conditions—**Because nitration reactions are sensitive to the presence of metal ions, the buffer solutions were exposed to air for 2 h in the dark at room temperature under the conditions of a fused silica capillary column (50-cm effective length, 75-μm internal diameter), hydrodynamic injection for 5 s, a temperature of 25 °C, voltage of 20 kV, normal polarity, 66 μA, and UV light detection at 280 nm. As an electrolyte solution, a mixture of 10 mM NaHPO4, 10 mM Tris, and 25 mM dodecyltrimethyl-ammonium bromide (pH 7.4) was employed. Migration time in capillary zone electrophoresis for NAT and NANT was 14 min (total time 16 min) in phosphate buffer (pH 7.4, 20 mM, ionic strength of 150 μM, pH 7.4).

The GSH-induced formation of NAT and the decay of NANT were additionally determined by either exciting the formed NAT (λexc = 270 ± 10 nm, λmax = 358 ± 10 nm, detection limit of <1 μM) or reading the optical density at 335 nm (ε335 (NANT) = 6100 M−1 cm−1) (13).

**Results**

**Formation of S-nitrosothiols—**To demonstrate the N-nitrosotryptophan-dependent formation of S-nitrosothiols, the lipophilic thiol NAP was mixed with 15N-NANT in Me2SO (Fig. 1, A–C). In this lipophilic environment the resonances of authentic 15N-NANT and preformed 15N-SNAP were found at 170 and 185 ppm (Fig. 1A) as well as at 455 ppm (Fig. 1B). In contrast to the experiments performed in phosphate buffer (see below), only three resonances were observed at 170, 185, and 455 ppm during the reaction of 15N-NANT with NAP (Fig. 1C).

That observation can be put down to the fact that NANT and SNAP are stable compounds in dried MeSO such that their decomposition products, nitrite and nitrate, did not accumulate. Because the formed 15N-SNAP exhibited a sharp and intense resonance line in MeSO, the NANT-dependent formation of the S-nitrosothiol via a transnitrosation reaction was unequivocally proven. Oae and Shinhama (16) suggested that S-nitrosothiols transnitrosate aromatic amines, but any evidence for such a reaction with an indole ring as the target is missing in the literature. As the 15N moieties of 15N-NANT was only partly transferred to the thiol group of NAP in MeSO even after a reaction period of 4 days (Fig. 1C), we wondered whether 15N-SNAP can transnitrosate NAT (Fig. 1D). In fact, after 6 days of incubation the characteristic resonances of 15N-NANT at 170 and 185 ppm appeared in the reaction mixture. Similar results were observed when NAT was replaced by melatonin (Fig. 1E). Again, the generation of 15N-nitrosomelatonin was unequivocally demonstrated by the increase of its 1H-NMR resonance lines at 166 and 178 ppm, respectively, after 6 days of incubation. Thus, in a non-aqueous solution the reaction of N-nitrosodiamine derivatives with thiols is in equilibrium with the products, as shown in Equation 6.

\[ {^{15}N}-NANT + \text{NAP} \rightleftharpoons {^{15}N}\text{-SNAP} \] (Eq. 6)

although the corresponding rate constants are obviously low.

To extend our data to the aqueous solution, the product pattern of the reaction of 15N-NANT (200 mM) with glutathione (150 mM) was analyzed by 1H-NMR-spectrometry. In phosphate buffer at pH 7.4, authentic GS15NO exhibited its 15N-NMR resonance line at ~390 ppm (Fig. 2A). An inspection of Fig. 2B and C demonstrated the occurrence of five 15N-NMR resonance lines during the reaction of 15N-NANT with GSH, i.e. 15N-nitrite at ~5 ppm, 15N-NANT at 167 ppm, 15N-nitrate at 182 ppm, and 15N-nitrate at 229 ppm. The spread and flattened resonance at 390 ppm belonged to the buildup of GS15NO.

Because the 15N-NMR resonance line of GS15NO may be affected by the aqueous solution, the formation of GSNO from the reaction of NANT (66 mM) with GSH (33 mM) was studied by UV-visible spectroscopy (Fig. 3A and B). In fact, the characteristic UV-visible absorption of GSNO at 545 nm continuously increased during the reaction of NAT and GSH (Fig. 3A), which is in full agreement with the 15N-NMR experiments. Noticeably, the reaction between NAT and the thiol was obviously orders of magnitude faster in water than in dried MeSO (see below). When equimolar concentrations of NANT and GSH were used, the UV-visible maximum at 545 nm did
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Fig. 1. Formation of SNAP, NANT and N-nitrosomelatonin in lipophilic medium. $^{15}$N-NMR analysis was employed at 25 °C in Me$_2$SO with authentic $^{15}$N-SNAP and $^{15}$N-NANT in the absence and the presence of either NAP or melatonin. A, 1 mM $^{15}$N-NANT. B, 250 mM $^{15}$N-SNAP. C, 1 mM $^{15}$N-NANT and 1 mM NAP. D, 1 mM $^{15}$N-SNAP and 800 mM NAT. E, 1 mM $^{15}$N-SNAP and 800 mM melatonin.
not increase continuously (Fig. 3B), probably because of a GS\(-\n\) induced depletion of S-nitrosoglutathione as reported by Singh et al. (17).

Influence of pH on the Yield of GSNO—As a possible consequence of the GS\(-\n\) -mediated decrease of S-nitrosoglutathione, the yield of GSNO at pH 7.4 leveled off to ~12% (Table I).

Because thiolate anions are expected to attack S-nitrosothiols, the NANT-dependent yield of GSNO should increase at more acidic pH values when the GS\(-\n\) -dependent side reaction cannot proceed effectively. Therefore, the pH was stepwise lowered down to 4.5, and the yields of GSNO were determined (Table I). In fact, decreasing the pH from 7.4 to 4.5 increased the yield of...
GSNO from ~12 to ~45%. Thus, the NANT-derived yield of GSNO at pH 7.4 is indeed affected by the GS -mediated decrease of the S-nitrosothiol.

Formation of NAT—In addition to GSNO, NAT should be formed during the reaction of NANT with GSH. To check this probability, the NANT/GSH (0.8 mM/5 mM)-dependent formation of NAT was established via capillary zone electrophoresis by spiking with authentic material at pH 7.4 (Fig. 4A, chromatograms a–c). The chromatograms clearly demonstrated the formation of NAT from the reaction of NANT with GSH. In parallel runs, the formed amount of NAT as well as the decreased concentration of NANT was distinguished by the fluorescence capabilities of NAT (λ_{ex} = 270 nm/λ_{em} = 358 nm) and the characteristic UV-visible absorbance of NANT at 335 nm (Fig. 4B, respectively. Fig. 4B clearly illustrates that the increase of the NAT concentration was strictly connected with the decrease of the NANT concentration during the reaction. For instance, the concentration of NAT increased by −0.4 mM during the reaction period, and about the same amount of NANT was denitrosated by GSH. However, we observed a continuous decrease of the total amount of NANT and NAT during the reaction. This tiny decrease is again explained by the GS -dependent side reaction. Reaction of low molecular weight S-nitrosothiols with thiols has been reported to yield nitroxyl (18), which generates strong oxidants like peroxynitrite (19), and tryptophan is a very good target for this peroxide and the radicals derived from it (20–22). Thus, the GSH-dependent denitrosation of NANT most likely yielded stoichiometric amounts of NAT, thereby fully restituting the indole ring during this transnitrosation reaction.

As expected from a true transnitrosation reaction, NANT was additionally denitrosated by GSH under hypoxic conditions at pH 7.4, but the backward reaction did not proceed because independently performed experiments demonstrated that the decay of GSNO (50 mM) was not altered in the presence of NAT (66 mM) (data not shown). Conclusively, the transfer of the nitroso function, shown in Equation 7,

\[ \text{NANT + GSH} \rightarrow \text{NAT + GSNO} \]  

(Eq. 7)

proceeded in aqueous solution only from the N-nitrosotryptophan derivative to the thiol.

Reaction Rate Constants—For a discussion of the putative significance of N-blocked, N-nitrosotryptophan-dependent nitrosation of thiols in vivo, the reaction between NANT and GSH was kinetically characterized. We refrained from determining the underlying rate constant \( k \) from the increase of the UV-visible absorbance of S-nitrosothiols at ~545 nm because of the GS -induced side reaction (see above). Because the absorbance coefficient of NANT (ε_{545} = 6100 M^{-1} cm^{-1}) is high, low concentrations of NANT (100 μM) and either GSH (100 μM) or cysteine (100 μM) were used for the determination of the rate constants. From the Arrhenius plots of the rate data (Figs. 5, A and B), activation barriers of 17.2/16 kcal and A-factors of 1.9e13/1.6e12 were extracted for GSH and cysteine, respectively (Table II). From the Arrhenius parameter we calculated the corresponding rate constants at 37 °C for the reaction of NANT with either GSH or cysteine and measured the rate constants of the similar reactions between N-nitrosomelatonin and these thiols (Table III). Interestingly, the rate constants of the transnitrosation reactions between N-terminal blocked tryptophan derivatives and thiols attained (more or less) a level that is evident for the transfer of the nitroso function from S-nitrosothiols to thiols.

DISCUSSION

In the present study we identified, as delineated in Equations 8 and 9,
a hitherto unknown transnitrosation reaction, i.e. the transfer of the nitroso function from N-nitrosotryptophan derivatives to thiols in aqueous and non-aqueous solutions.

The finding that, via a transnitrosation reaction, S-nitrosothiols can generate N-acetyl-N-nitrosotryptophan as well as N-nitrosomelatonin in Me$_2$SO, i.e. the backward reactions of Equations 8 and 9, is a second novelty. On the other hand, such a spontaneous backward reaction is very slow in non-aqueous solutions and does not spontaneously proceed in an aqueous solution. This fact may strongly limit its impact under physiological conditions, but because quantum-chemical calculations predict that the thermodynamical equilibrium lies on the side of the N-nitrosotryptophan derivatives, the importance of the backward reactions might strongly increase when a yet unidentified enzymatic catalysis is operating in vivo. However, in an aqueous solution the spontaneous forward reaction (Equations 8 and 9) is strongly favored so that the reaction rate constants reach values that are comparable with the ones known for the transfer of the nitroso function from S-nitrosothiols to thiols (Table III). Noticeably, transnitrosation reactions between S-nitrosothiols and thiols are now believed to be involved “in the transport and targeting of NO bioactivity in signal transduction” (23) and “in membranes for transfer of NO” (23, 24). Thus, the rate constants of the transnitrosation reaction between N-nitrosotryptophan derivatives and thiols are obviously high enough to achieve some importance under physiological conditions.

Previous studies have shown, as seen in Equation 10,

$$\text{NANT} + \text{H}_2\text{O} + \text{H}_2\text{O}^+ \rightarrow \text{NAT} + \text{H}^+ + \text{NO}_2^-$$

(Eq. 10)

that the hydrolysis of NANT at 37 °C and pH 7.4 limits its maximal half-life time to −140 min (10). Because an identical half-life time was found for nitrosated tryptophan in human serum albumin (25), it is to be expected that the hydrolysis of other N-nitrosotryptophan derivatives would proceed at a similar speed. From this point of view, the lifetime of N-nitrosotryptophan derivatives seems to be long enough for operating as an intermediate in vivo. The transfer of the nitroso function to thiols must, of course, decrease the lifetime of N-nitrosotryptophan derivatives, and the question arose about physiological N-nitrosotryptophan concentrations in plasma. Because N-nitroso compounds were found in aortic rat tissues (26) and proline can be nitrosated only at acidic pH values (27, 28), one may conclude that N-nitrosotryptophan derivatives represent the major amount of the experimentally identified N-nitroso compounds in vivo. The major amount of N-nitrosotryptophan derivatives would be located in proteins, because the concentrations of low molecular weight, N-terminal blocked tryptophan derivatives like melatonin are very low in plasma (<0.2 nM) (29). The main mass thiols circulating in blood are albumin (680 μM) (6), cysteine (~10 μM) (30), homocysteine (5–15 μM) (31), and glutathione (7 μM) (30). Assuming that one reaction partner of the transnitrosation reaction should be a low molecular weight molecule and keeping in mind the plasma concent-

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2 M. Kirsch and H. de Groot, unpublished results.
A frequency factor, and render the steady-state plasma concentration of N-nitroso compounds to thiols would outcompete the hydrolysis reaction at N-nitrosotryptophan concentrations >25 nM. In other words, the low molecular weight thiols are expected to render the steady-state plasma concentration of N-nitrosotryptophan derivatives to an upper limit of ~25 nM, which is in full agreement with the experimental value of 33 ± 6 nM for the total amount of N-nitroso compounds in aortic rat tissues (26).

In 1996 Zhang et al. (32) studied the biological activity of N-nitrosated Gly-Trp and carboxymethyl bovine serum albumin (CM-BSA), a modified derivative in which the thiol group is covalently blocked, in comparison with GSNO. Because these data were a bit distorted by the nitroso content of the applied nitroso compounds, i.e. 89, 22, and 19% for GSNO, Gly-NO-Trp, and N-nitroso CM-BSA, respectively, we approximated the data of Zhang et al. to a nitroso content of 100% (Table IV). Interestingly, the corrected data clearly demonstrate that the efficacy of the Gly-NO-Trp-induced vasorelaxation and the inhibition of platelet aggregation was nearly identical to that of GSNO. Because Gly-NO-Trp does not spontaneously diffuse through cellular membranes, Zhang et al. (32) suggested that extracellular N-nitroso-compounds transfer the nitroso function via a transnitrosation reaction to a NO-carrier “located on or associated with the plasma membrane” (32). Assuming that a protein-bound cysteine is the NO carrier, which can indeed be highly effective in transporting nitric oxide through cellular membranes (23, 24), the small peptide Gly-NO-Trp may induce such a process. However, a N-nitroso CM-BSA-dependent transnitrosation reaction of a protein-bound cysteine at the plasma membrane seems to be highly unlikely because of a massive steric hindrance during such a transfer of the nitroso function. Thus, a reason other than a N-nitroso CM-BSA-dependent transnitrosation of the membrane-bound cysteine must be found to explain the observed capabilities (Table IV). It should be noted that the experiments of Zhang et al. (32) were performed either in the presence of phentolamine, as in the case of the vasorelaxation activity experiments, or (presumably) in the presence of aspirin, as in the case of the platelet aggregation experiments. Because catecholamines and ascorbate (10) release nitric oxide from N-nitrosotryptophan compounds, the N-nitroso CM-BSA-dependent effects of vasodilation and anti-coagulation were obviously thiol-independent.

However, it may be a pharmacological improvement to stimulate the reaction of low molecular weight, N-terminal blocked N-nitrosotryptophan derivatives with the plasma thiols by applying NANT or derivatives of it. The mutagenicity of NANT in bacteria as described by Venitt et al. (33) only occurred at concentrations >180 μM, and harmful effects were not observed at typical pharmacological concentrations (~1–50 μM) (34). These findings should not limit the use of NANT or derivatives of it as a pharmacological compound, because a variety of prodrugs induce toxicological pathways at higher concentrations, e.g. the drug sodium nitroprusside (35). Proving that NANT is a harmless compound at low plasma concentrations

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**TABLE II**

Arrhenius parameters for the reactions between NANT with either GSH or cysteine

| Reaction | Arrhenius parameters | NANT + GSH | NANT + cysteine |
|----------|----------------------|-------------|-----------------|
| A        | 1.9 ± 0.3E + 13      | 1.6 ± 0.3E + 12 |
| E_a      | 72 kJ ± 1.7         | 67 kJ ± 1.7  |
| (15.2 kcal ± 0.4) | (16 kcal ± 0.4)     |

**TABLE III**

Rate constants for the transfer of the nitroso-function from nitroso-compounds to thiols

| Reaction | kᵢ | Source | Temperature |
|----------|----|--------|-------------|
| NANT + GSH | 13.1 ± 2.5 | This paper | 37 |
| NANT + cysteine | 5.9 ± 0.9 | This paper | 37 |
| NO-mela* + GSH | 13.5 ± 5.6 | This paper | 37 |
| NO-mela* + cysteine | 7.2 ± 2.4 | This paper | 37 |
| SNAP + GSH | 9.09 ± 0.78 | 48 | 37 |
| SNAP + cysteine | 20.63 ± 3.15 | 48 | 37 |
| GSNO + NAP | 0.53 ± 0.13 | 48 | 37 |
| CSNNO + NAP | 3.15 ± 1.11 | 48 | 37 |

* Melatonin.

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**TABLE IV**

Bioactivity of nitroso-compounds

| Nitroso compounds | Vasorelaxation activity | Inhibition of platelet aggregation |
|-------------------|-------------------------|----------------------------------|
|                   | Observed | Corrected | Observed | Corrected |
| GSNO              | 0.43     | 0.38      | 0.7      | 0.62      |
| Gly-NO-Trp        | 1.13     | 0.25      | 3.5      | 0.77      |
| N-nitroso CM-BSA  | 15      | 2.85      | 17.3     | 3.12      |

* EC₅₀, † IC₅₀, ‡ IC₅₀.
(≤20 μM), there may be several advantages for its use as compared with a suggested application of low molecular weight S-nitrosothiols. For example, exogenous S-nitrosothiols operate as vasodilators in vivo (36), and GSNO was found to be 10-fold more potent in arterioles than in veins (40). However, low molecular weight S-nitrosothiols do not react regiospecifically with thiols because, as shown in Equation 11,

\[ R'S(H) + R'SNO \rightarrow R'SSR + (H)NO \]  

(Eq. 11)

they generate the harmful (41, 42), nitric oxide-scavenging (43) compound nitrotyrosyl in a prominent side reaction (18, 44).

Knowledge about the precise relative rates of these two reactions is very tenuous, but it is clear that a relative slow reaction (Equation 11) is not effectively suppressed when the competition reaction, shown in Equation 12,

\[ R'S(H) + R'SNO \rightleftharpoons R'SNO + R'S(H) \]  

(Eq. 12)

is a reversible one. Thus, although exogenous S-nitrosothiols are vasodilators, their pharmacological activity is presumably a bit hampered by the reaction outlined in Equation 11. This drawback will not be present with the application of NANT, because N-nitrosotryptophan derivatives regiospecifically transnitrosate thiols, thereby generating the harmless compound N-acetyltryptophan and a vasodilatative active S-nitrosothiol (Equation 8). In addition, one may suggest applying NANT as a mainly nitric oxide-releasing drug by co-administrating ascorbate. However, we believe that the suggested application of N-nitrosomelanotin (45) cannot be a pharmacological improvement, because in such a case melatonin would be generated at unphysiological high concentrations, thereby supporting harmful side reactions like abdominal pain and hypotension (46, 47).

CONCLUSIONS

The novel S-nitrosothiol-yielding reaction between N-nitrosotryptophan derivatives and thiols is fully characterized. The transfer of the nitroso function from the N-nitrosotryptophan derivative to the thiols proceeds with rate constants that are very typical for S-nitrosothiol-dependent transnitrosation reactions. The spontaneous formation of S-nitrosothiols from the reaction of thiols with N-nitrosotryptophan derivatives is irreversible in aqueous solution, which would limit the steady-state concentrations of the latter nitroso-compounds in biological systems. However, this irreversible transnitrosation reaction may be of pharmacological interest in order to stimulate the formation of S-nitrosothiols in vivo by applying low molecular weight N-nitrosotryptophan derivatives like NANT.

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