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Evaluation of transnitrosating ability of *N*-nitrosoguanidines to alkyl thiols and thiol amino acids

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ABSTRACT

The transfer of the nitroso group from 1-nitroso-1-methyl-3-tolylsulfonylguanidine (NOTSG) and 1-nitroso-1-methyl-3-benzoylguanidine (NOBMG) to some thiols, including the amino acid cysteine, was studied in a pH range between 7 and 12.

The measured apparent bimolecular rate constant of transnitrosation (k_{tr}^{app}) revealed a bell-shaped pH dependence that clearly indicates that both nitrosoguanidines react through the corresponding neutral form, and the nucleophiles in the thiolate anion form to give the corresponding *S*-nitrosothiol. Regarding cysteine, the existence of three macroscopic acidity constants influenced the kinetic behavior of the transnitrosation reaction. Transnitrosation rates (k_{tr}) of the two possible nucleophilic species were obtained and it was found that NOBMG has lower thiol transnitrosation capacity due to the lower electron-withdrawing effect of benzoyl group and to the possible stabilization of the anionic structure as a consequence of the establishment of the intramolecular hydrogen bond. The k_{tr} values of the studied nucleophiles were calculated and a Brønsted-type plot was established giving unexpected negatives β_{nuc}(β_{nuc}(NOBMG)=−0,17 and β_{nuc}(NOTSG)=−0,11). The atypical β_{nuc} values were attributed to the need for previous desolvation of the nucleophile.

1. Introduction

The discovery of physiological properties of nitric oxide (NO), particularly those of vasodilation and inhibition of platelet aggregation,[1–3] is actually responsible for the major interest in the chemistry of nitrosothiols (RSNOs).

Several RSNOs like *S*-nitrosocysteine, *S*-nitrosoglutathione, *S*-nitrosoalbumins and *S*-nitrosohemoglobin, have been detected in vivo[4] and are believed to be responsible for the NO transport and release around the body.[5–7]

RSNOs are very readily generated in solution by nitrosation of the corresponding thiols using conventional nitrosation methods[8] or transferring the nitroso group from a nitrosocompound to thiolate ions (transnitrosation).[9–11]

From all RSNOs with biological relevance, *S*-nitrosocysteine is probably one of the most important and therefore this *S*-nitrosothiol has been the aim of several mechanistic studies concerning its decomposition with simultaneous NO generation.[4,8,11–13]

However, there is little information about *S*-nitrosation using transnitrosating ability of nitrosocompounds in the presence of sulfur nucleophiles such as cysteine (Cys). Only two mechanistic studies about MNTS reactivity in the presence of cysteine, at several pH values, were found in the literature that showed the *S*-nitrosocysteine obtention by a transnitrosation process.[9,14]

It is known that *N*-nitrosocompounds, like MNTS (Fig. 1), have a high cytotoxic activity due the possible formation of electrophilic species by a deamination process[15] and the possible transfer of the nitroso group to amines increases the risk of carcinogenicity of these compounds,[16] however, in the presence of sulfur nucleophiles

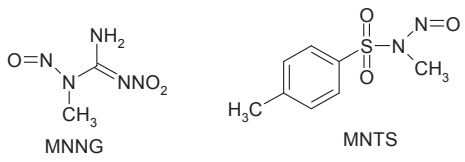

Fig. 1. *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and *N*-methyl-*N*-nitroso-*p*-toluenosulfonamide (MNTS) structures.

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usually more reactive than amines, these compounds can assume a new and interesting role as NO donors.

Due to this duality, our group has been interested in the chemistry and reactivity of some *N*-nitrosocompounds, particularly nitrososulfonamides, nitrosothioureas and nitrosoguanidines. After confirming the ability of these compounds to transnitrosate to amines[15,17–19] and based on some studies realized with MNTS[9,14] and MNNG[20] (Fig. 1) involving nitric oxide and *S*-nitrosocysteine formation, it was our goal to study the nitroso group transfer from two *N*-nitrosoguanidines containing electro-withdraw groups (NOTSG and NOBMG) to thiolate ions including cysteine.

To accomplish our purpose, the transnitrosation ability of NOBMG and NOTSG to sulfur nucleophiles was kinetically evaluated, first with thioles containing only the thiol function with ionization capacity, and then with cysteine an amino acid that in addition to the thiol function has two other ionizable functions: α-carboxilate and α-amino.

The thioles used (Table 1) were chosen based on their basicity. The complete kinetic study was accomplished using the nucleophile 2-mercaptoethanol (ME).

Table 1
pK_a values of studied sulfur nucleophiles (RSH)

RSH	Trivial name	pK_a^{RSH}
$CH_3O_2CCH_2SH$	Methylhtioglycolate (MTG)	7,81[21]
$CH_3O_2CCH_2CH_2SH$	Methyl 3-mercaptopropionate (MMP)	9,33[22]
$OHCH_2CH_2SH$	2-mercaptoethanol (ME)	9,60[22,23]
CH_3CH_2SH	Ethanothiol (EtSH)	10,6[21]

2. Results and discussion

2.1. Preliminary analysis of the transnitrosation products

The transnitrosating ability of *N*-nitrosoguanidines (Fig. 2) to some thioles at different pH values was previously studied under similar conditions of those described for the kinetics studies developed in this work.

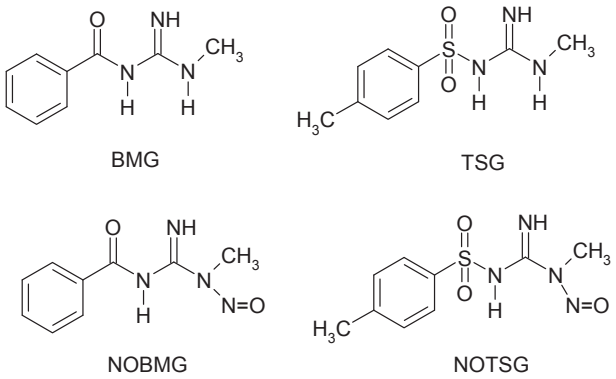

Fig. 2. Free guanidines structures: 1-methyl-3-benzoylguanidine (BMG) and 1-methyl-3-tolylsulfonylguanidine (TSG) and their correspondent *N*-nitrosated derivatives: 1-nitroso-1-methyl-3-benzoylguanidine (NOBMG) and 1-nitroso-1-methyl-3-tolylsulfonylguanidine NOTSG).

The obtained reaction mixtures were treated as described in the experimental section and analyzed by TLC that showed the denitrosated guanidines BMG and TSG (Fig. 2), respectively, as the principal final products of these reactions.

In the reactions performed at pH≥11 it was also detected the presence of benzoic acid, or *p*-toluenesulfonic acid if the nitrosating agent was NOBMG or NOTSG.

The presence of free guanidines BMG and TSG is consistent with the possible direct transfer of nitroso group to the thioles, and the acid benzoic (or *p*-toluenesulfonic acid) anions formation at pH≥11 is in agreement with the competition from basic hydrolysis of nitrosoguanidines.[24]

2.2. Transnitrosation to simple thioles (RSH type)

The influence of ME concentration on the observed rate constants, k_{obs}, of its reaction with the *N*-nitrosoguanidines was studied at different pH values. Figs. 3 and 4 illustrate the good linear

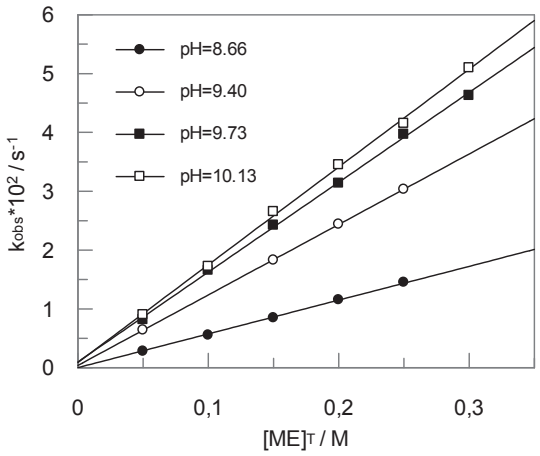

Fig. 3. Influence of 2-mercaptoethanol total concentration on k_{obs} in transnitrosation reaction with NOBMG at 25 °C.

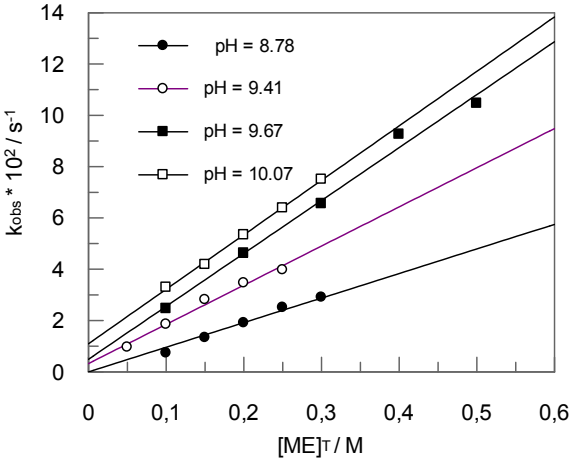

Fig. 4. Influence of 2-mercaptoethanol total concentration on k_{obs} in transnitrosation reaction with NOTSG at 25 °C.

relationship with a zero intercept between k_{obs} and the total thiol concentration, $[ME]_T$, indicating the absence of NOBMG (or NOTSG) basic hydrolysis competition.[9]

It is also evident, for both *N*-nitrosoguanidines, the slope increases with the increasing pH, which is consistent with the Eq. 1, where k_{tr}^{app} is an apparent bimolecular rate constant since it includes the different acid-base equilibrium constants of the nucleophile and, $[RSH]_T$ represents in this case the total concentration of ME.