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Nitroso Group Transfer in *S*-Nitrosocysteine: Evidence of a New Decomposition Pathway for Nitrosothiols

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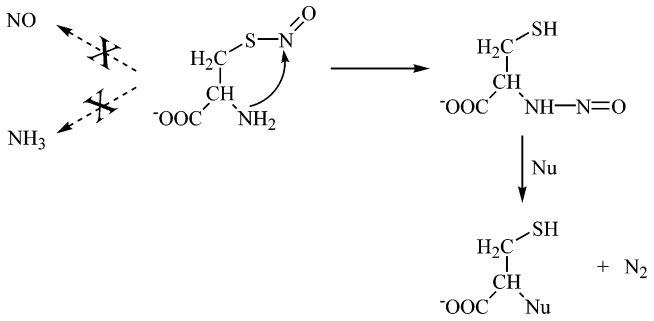
Received April 21, 2005



The rate of *S*-nitrosocysteine decomposition in a pH range between 0.7 < pH < 13 exhibits first- and second-order dependence on total cysteine concentration. The second-order term is only observed for pH values between 6.9 < pH < 12. Both first- and second-order terms show a complex dependence on the acidity of the medium. They increase with increasing pH, reaching a maximum value around pH = 8 and then decrease with further increase in pH. An analysis of the reaction products reveals the absence of nitrite ion and ammonia. No evidence of catalysis by copper ions is observed. These results suggest the existence of a new decomposition pathway for *S*-nitrosocysteine, which proceeds via an intramolecular nitroso group transfer producing a primary *N*-nitrosamine that decomposes rapidly to give the corresponding diazonium salt. The nitroso group transfer reaction occurs intermolecularly for the decomposition pathway exhibiting a quadratic dependence on cysteine concentration. Both nitroso group transfer pathways are subject to acid catalysis by cysteine. Kinetic results indicate that the extent of S···NO bond cleavage in the transition state is ahead of protonation of the AH···S sulfur atom. The results obtained show the existence of a new decomposition pathway for the *S*-nitrosocysteine where NO is not released, and hence, it has a significant biological impact due to the potential use of nitrosothiols as NO donors.

Introduction

Most chemists are now aware of the series of spectacular developments that occurred from 1988 onward when it was discovered that nitric oxide is synthesized in vivo from L-arginine and that it is involved in a wide range of physiological functions as diverse as vasodilation, inhibition of platelet aggregation, neurotransmission, and penile erection together with having a major role to play in the operation of the immune system. These major discoveries have sparked off massive research efforts in the biology and chemistry of nitric oxide, and new developments regarding further involvement of nitric oxide in vivo are being made continually.[1] It is important to point out that a thematic issue of *Chemical Reviews* has been devoted to illustrate the diverse aspects of NO chemistry in a modern context in which the role of NO as a physiological signal and metabolic intermediate is paramount, covering important areas of NO chemistry from physical and computational studies to the biological role in living systems.[2] Due to the instability and inconvenient handling of aqueous solutions of authentic NO, there is an increasing interest in using compounds

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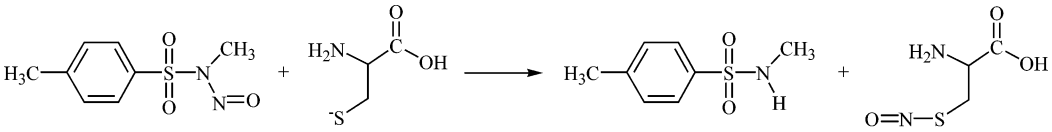
10.1021/jo050811r CCC: $30.25
Published on Web 07/07/2005

SCHEME 1



MNTS

capable of generating NO in situ, that is, NO donors. The most widely used NO donor is glyceryl trinitrate (nitroglycerine).[3] In addition to organic nitrates, many other chemicals can be transformed into NO in vitro or in vivo. Due to the diversity of NO donor structures, the pathway for each class of compounds to generate NO could differ significantly, for example, enzymatic versus nonenzymatic, reductive versus oxidative, and so forth. Because each class of compound offers distinct biochemical properties, it allows one to choose a compound that best meets the demands of specific investigations. All nitrogen−oxygen-bonded compounds have the potential to decompose, be oxidized, or be reduced to produce reactive nitrogen species. Major classes of current NO donors along with their individual pathway of NO generation have been summarized.[4] In the nonenzymatic pathway of NO generation from organic nitrates (RO−NO_2), organic nitrites (RO−NO), metal−NO complexes, nitrosimines, nitrosothiols, diazetine dioxides, furoxans, benzofuroxans, and oxatriazole-5-imines, thiols represent a main cofactor for NO releasing. In these nonenzymatic pathways *S*-nitrosothiol is generated,[5] from which there is a ready pathway to NO formation by a Cu^{2+}-catalyzed reaction. Other nitroso compounds can react with thiolate ions generating *S*-nitrosothiols, as in the case of *N*-nitrososulfonamides[6] or *N*-nitrososulfonilguanidines.[7]

In this sense, the enormous interest aroused by the chemistry, biochemistry, and physiology of the sulfur analogues of alkyl nitrites, *S*-nitrosothiols (RSNO), in the past few years is readily explained. The reason for this interest arises because as a class they have been found to have much the same physiological properties as NO itself, particularly of vasodilation[8] and of the inhibition of platelet aggregation.[9] They have also been identified in bodily fluids, notably as *S*-nitrosoglutathione[10] and *S*-nitrosoalbumins.[11] Indeed, the current belief[12] is that NO is transported around the body as RSNO (mostly as the nitrosoalbumins), from which NO can be released under certain conditions. This belief derives mostly from the fact that the measured half-life of NO itself in vivo is very short (estimates range from a few seconds to a few minutes), whereas RSNO species are generally much more stable in solution.

S-Nitrosothiols are very readily generated in solution by conventional nitrosation of thiols, and examples have been known for about 100 years.[13] In contrast with the corresponding nitrosation of alcohols by sodium nitrite in mildly acidic solution, the equilibrium position corresponding to thiol nitrosation lies well over the right, with equilibrium constant[14] of 10^5-10^6 M^{-1}. This difference between ROH and RSH has been rationalized in terms of the different nucleophilicities of the O and S atoms in the alcohols and thiols and the different basicities of the same atoms in the alkyl nitrites and *S*-nitrosothiols. Nitrosation of thiols has been examined mechanistically and follows the pattern of amine nitrosation, in which both acid- and halide ion catalysis occur.[15] In principle, any other nitrosating agent (such as an alkyl nitrite) will also bring about reaction in nonaqueous solution or in basic media if necessary. However, in contrast to the alkyl nitrites, relatively few *S*-nitrosothiols are stable in the pure form. *S*-Nitrosothiols decompose thermally and photochemically to give the disulfide and initial nitric oxide. For some, the thermal reaction is clearly very slow at room temperature (solid SNAP has to be heated to about 150 °C before significant decomposition occurs). In many other cases (e.g., *S*-nitrosocysteine), the decomposition rate in the pure solid state is much higher, not allowing isolation.

This article discusses the stability of *S*-nitrosocysteine over a wide pH range under conditions where *S*-nitrosocysteine coexists with an excess of thiol and thiolate. *S*-Nitrosocysteine has been generated by reaction with *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (MNTS, Scheme 1) using the nitrosating agent as the reactant in deficiency. Experimental results allow us to propose a new decomposition pathway for *S*-nitrosocysteine through an intramolecular nitroso group transfer. The main characteristic of this new decomposition pathway is the absence of nitric oxide as a reaction product, a fact that provides important biological repercussions. The existence of this new decomposition pathway for *S*-nitrosocysteine would explain why NO could not be detected from the reaction of nitroglycerine with cysteine using the very sensitive NO-specific electrode method.[1d]

Experimental Section

All reagents were products of the highest purity commercially available and used without further purification.

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Kinetic experiments were carried out under different conditions depending on the pH range considered. Cysteine buffer solutions were used for pH values between $6.89 < pH < 11.30$. To prepare *S*-nitrosocysteine, the cysteine buffer [buffer] = (0.025−0.30) M was made to react with MNTS, [MNTS] = 5.00×10^{-4} M. Under these conditions, the *S*-nitrosation reaction of cysteine occurs within a few seconds[16] with the formation of the *S*-nitroso compound being observed at $\lambda = 330$ nm. The decomposition reaction of the *S*-nitroso compound was subsequently studied in the presence of the cysteine buffer itself. For pH values between 12 and 13, the acidity of the reaction medium was controlled by the addition of the corresponding NaOH concentration. In these cases, the cysteine concentration was varied between [cysteine] = (0.05−0.30) M.

For pH values between $0.9 < pH < 3.11$, the formation reaction of the *S*-nitroso compound is very slow, and it competes significantly with its decomposition in such a way that variation in absorbance is hardly observed at $\lambda = 330$ nm. To solve this problem, *S*-nitrosocysteine is generated at pH = 12 using approximately equimolar concentrations of cysteine and MNTS. After a few seconds, suitable quantities of HCl and cysteine are added to the reaction medium to get the desired pH and [cysteine]. Kinetic studies were carried out using H_2PO_4Na/HPO_4Na_2 ($5.03 < pH < 6.07$) and CH_3COOH/CH_3COONa (pH = 3.29 and pH = 3.72) buffers. In both cases *S*-nitrosocysteine was generated at pH = 12, and appropriate quantities of buffer and cysteine were subsequently added to achieve the desired experimental conditions. In these cases, the cysteine concentration was varied between [cysteine] = (0.006−0.03) M, keeping the buffer concentration constant, [buffer] = 0.25 M.

Due to the low solubility of MNTS in water, its solutions were prepared in an acetonitrile/water mixture, in such a way that the percentage of acetonitrile in the reaction mixture was 1% (v/v). The pH was measured with a pH meter equipped with combined glass electrodes. Kinetic studies were always carried out at 25 °C and constant ionic strength of the reaction medium (I = 1.00 M, $NaClO_4$). Kinetic experiments were carried out in a UV−vis−NIR spectrophotometer monitoring the decreasing absorbance at $\lambda = 330$ nm due to disappearance of thionitrite. Good first-order behavior was found throughout, following every run to at least 90% reaction. Fitting the absorbance−time data to the first-order integrated rate equation allows us to obtain the pseudo-first-order rate constants, k_{obs}, used in the subsequent analysis. Values of k_{obs} are subject to a standard error of 3%.

Nitrite ion was determined using a modification of Shinn's method.[17] The reaction mixture was brought to pH ≈ 2 and mixed with sulfanilamide and naphthylethylenediamine. Absorbance at 550 nm of the dye formed was measured, and nitrite ion was quantified. The existence of ammonia as reaction product was determined by the Nessler[18] reagent and by measuring absorbance at $\lambda = 425$ nm.

Results

When *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide is added to a cysteine solution, two clearly distinct kinetic processes can be observed at $\lambda = 330$ nm. The first process occurs on a time scale of seconds, and it leads to the formation of *S*-nitrosocysteine,[16] as can be inferred from the change in absorbance observed at 330 nm. The second process, subject of the present study, shows the disappearance of *S*-nitrosocysteine. This second process has been studied in a pH range between $0.7 < pH < 13$ using HCl, acetate buffers, phosphate buffers, cysteine buffers, and NaOH to keep constant the acidity of the medium. The observed kinetic behavior differs according to the pH range considered.

The possibility that the reaction might be subject to copper ion catalysis was also considered. For this purpose, the decomposition rate of *S*-nitrosocysteine was compared in the absence and the presence of EDTA, [EDTA] = 2×10^{-4} M. The results obtained show that the observed rate constant is not affected by the presence of EDTA, which indicates the absence of copper ion catalysis. This result suggests that the pathway of copper ion-catalyzed[19] decomposition is not effective under our experimental conditions. Another decomposition pathway, leading to thiol and nitrous acid, involves loss of NO^+ rather than NO and occurs with Hg^{2+} and, to a lesser extent, with Ag^+.[20] As will be discussed later, the absence of nitrite ion as the reaction product also allows the possibility of decomposition catalyzed by Hg^{2+} or Ag^+ to be excluded.

Oxidation of NO by oxygen to give nitrogen dioxide is a well-known reaction in water, which is unaffected by pH in the range 1−13.[21] The final product in water is exclusively nitrite ion, with very little if any nitrate ion. We have determined nitrite ion or nitrous acid using the well-known Shinn method, which allows us to detect nitrite concentrations of about 10^{-6} M. Considering the *S*-nitrosocysteine concentration generated by reaction with MNTS and the accuracy of the Shinn method, we can affirm that we are in a position to detect nitrite concentrations corresponding to 0.2% of the reaction. However, no significant amounts of nitrite could be detected under any of the experimental conditions used for the kinetic studies. Our kinetic studies have been conducted under aerobic conditions, in such a way that the NO that could be generated from the decomposition of *S*-nitrosocysteine ought to be quantitatively transformed into nitrite ion, as demonstrated by Williams[19a] et al. for the decomposition of *N*-acetyl-*S*-nitroso-penicillamine. Thus, the absence of nitrite ion indicates the absence of NO and nitrous acid as reaction products.

The absence of nitrous acid excludes the possibility of the decomposition of *S*-nitrosocysteine occurring by an acid hydrolysis process.[22] This process only takes place if the nitrous acid is removed (as the reaction lies well over toward the *S*-nitrosothiol) and also only at very high acidity, typically (2−3) M H_2SO_4. Similarly, the absence of NO as the product of the decomposition reaction of *S*-nitrosocysteine excludes the possibility that this process corresponds to the thermal decomposition or photodecomposition, since NO would be generated in both

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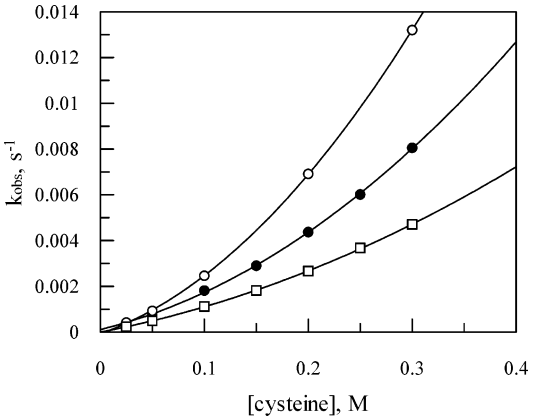

FIGURE 1. Influence of cysteine concentration on k_{obs} in the decomposition of *S*-nitrosocysteine at 25 °C. (●) pH = 7.14, (○) pH = 7.96, and (□) pH = 9.38.

cases. In addition, the metal ion-catalyzed decomposition can be discarded on the basis of the absence of copper ion catalysis and of nitrite ion as a reaction product.

Under our experimental conditions, we have determined the presence of ammonia using the Nessler[18] method. The accuracy of the method enables us to detect quantitatively amounts of ammonia corresponding to 2% of the decomposition of *S*-nitrosocysteine. However, the obtained results indicate a clear absence of NH_3, allowing us to exclude the decomposition pathway of nitrosothiols yielding NH_3 instead of NO.[23] Experiments in which a 2×10^{-3} M *S*-nitrosocysteine concentration was generated were carried out with a view to detecting lower reaction percentages that could lead to the formation of ammonia. Measurable quantities of NH_3 have not been detected under these conditions, in such a way that we can conclude that the ammonia concentration that may be formed in all cases must be less than 0.4%.

Figure 1 illustrates the influence of cysteine buffer concentration on k_{obs} for the decomposition of *S*-nitrosocysteine in a pH range between 6.9 < pH < 12. As can be seen, there is a linear and quadratic dependence of the observed rate constant on cysteine. In addition, the quadratic dependence of k_{obs} on [cysteine] is observed to be maximum at pH values near pH = 8 and to decrease with both increasing and decreasing pH.

Experimental results were fitted to eq 1.

$$k_{obs} = k_1^{app}[\text{cysteine}] + k_2^{app}[\text{cysteine}]^2 \qquad (1)$$

where k_1^{app} and k_2^{app} carry the dependence on the acidity of the medium.

Figure 2 shows the kinetic behavior observed both in the presence of H_2PO_4Na/HPO_4Na_2 buffers and when HCl is used to keep the acidity of the reaction medium constant. A linear dependence of k_{obs} on cysteine concentration is observed, with no sign of a quadratic term (eq 2):

$$k_{obs} = \text{intercept} + k_1^{app}[\text{cysteine}] \qquad (2)$$

In the presence of an external buffer (H_2PO_4Na/HPO_4Na_2 or CH_3COOH/CH_3COONa), the existence of a nonzero intercept corresponding to the rate of the buffer-catalyzed decomposition of *S*-nitrosocysteine is observed, and it will be the subject of future research.

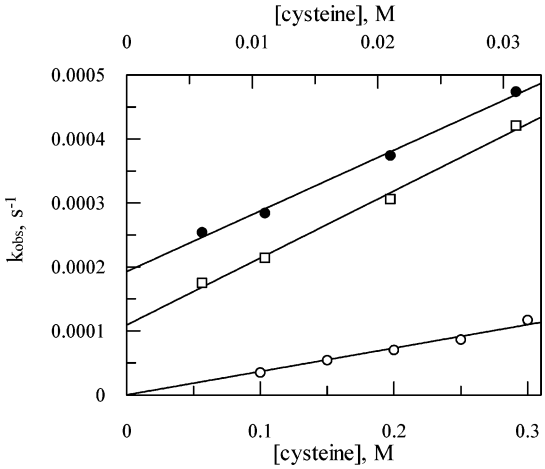

FIGURE 2. Influence of cysteine concentration on k_{obs} in the decomposition of *S*-nitrosocysteine at 25 °C. (○) pH = 2.08 (HCl), (●) pH = 5.03 (buffer H_2NaPO_4/HPO_4Na_2), and (□) pH = 6.07 (buffer H_2NaPO_4/HPO_4Na_2).

From Figures 1 and 2, it can be concluded that both the linear term, k_1^{app}, and the quadratic term, k_2^{app}, display a complex dependence on the acidity of the medium. Figures 3 and 4 show the influence of the acidity on these terms. As can be observed, k_1^{app} increases with the pH of the medium, reaching a limiting value at pH ≈ 6. A new increase of k_1^{app} occurs as the pH is increased, reaching a maximum value at pH ≈ 8. Further increases in pH result in a decrease of the k_1^{app} value. The pH dependence of k_2^{app} is likewise complex. The k_2^{app} term is not kinetically detectable either at pH values < 6 or at pH values > 12. In the acidity range between pH = 6 and pH = 12, the k_2^{app} value is observed to increase, with pH reaching a maximum value at pH value ≈ 8. A subsequent decrease in the acidity of the medium will cause a decrease in k_2^{app}.

Discussion

The absence of nitrite ion and NH_3 as the reaction products as well as the absence of Cu^{2+} catalysis and evidence against acid hydrolysis of *S*-nitrosocysteine indicate that the decomposition reaction should take place through a nitroso group transfer. RSNO compounds are able to nitrosate amines to form nitrosamines.[24] These transnitrosation reactions have been shown to occur readily in aqueous solution at pH ≥ 7.4.[25] The reactions of RSNO with thiols to give unsymmetrical

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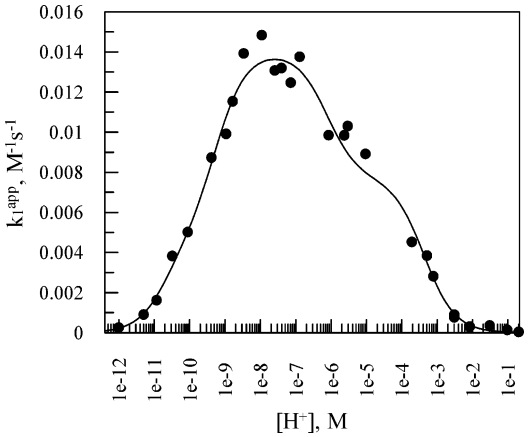


FIGURE 3. Influence of $[H^+]$ on k_1^{app} in the decomposition of *S*-nitrosocysteine at 25 °C and I = 1.00 M ($NaClO_4$). The acidity of the medium was controlled using HCl, CH_3COOH/CH_3COONa buffers, H_2PO_4Na/HPO_4Na_2 buffers, buffers of cysteine itself, and NaOH. The solid line represents the fit of experimental k_1^{app} values to eq 6.

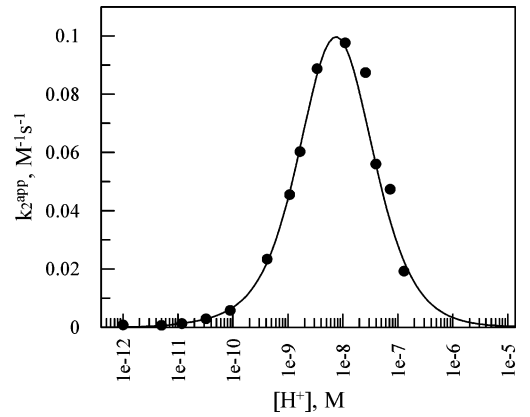
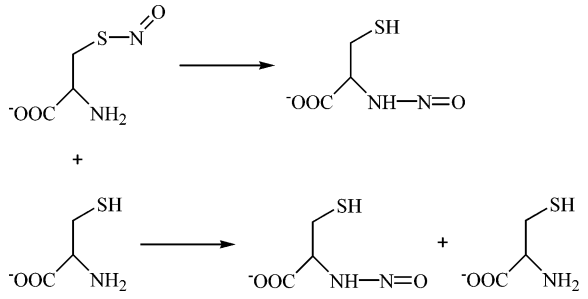


FIGURE 4. Influence of $[H^+]$ on k_2^{app} in the decomposition of *S*-nitrosocysteine at 25 °C and I = 1.00 M ($NaClO_4$). The acidity of the medium was controlled using HCl, CH_3COOH/CH_3COONa buffers, H_2PO_4Na/HPO_4Na_2 buffers, buffers of cysteine itself, and NaOH. The solid line represents the fit of experimental k_2^{app} values to eq 8.

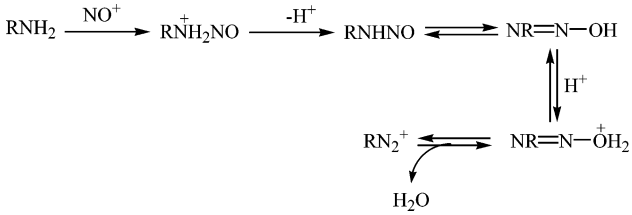
disulfides (RSSR′) were reported by Oae.[26] It has also been shown that, at high thiol concentrations, the reaction between RSNO and thiols (first-order in both reactants) results in the formation of the disulfide and ammonia as the principal final products, and the reaction was found not to be catalyzed by metal ions.[23] In our experimental conditions, the possible transnitrosation reaction from *S*-nitrosocysteine to cysteine cannot be responsible for the disappearance of RSNO since we would be in condition of an identity reaction. In addition, the absence of ammonia in the reaction medium allows us to eliminate the possibility that the decomposition of *N*-nitrosocysteine is due to the nitroso group transfer reaction from *S*-nitrosocysteine to the sulfur atom of another cysteine molecule.

Another potential decomposition pathway for *S*-nitrosocysteine is the intramolecular and intermolecular

SCHEME 2



SCHEME 3



nitroso group transfer reaction from *S*-nitrosocysteine to the nitrogen atoms of *S*-nitrosocysteine itself (intramolecular) or of another cysteine molecule (intermolecular) (Scheme 2).

The reaction will result in the formation of *N*-nitrosocysteine. Primary amines react readily with nitrosating agents to give products of deamination. The intermediates, primary nitrosamines (RNHNO), are not stable; therefore, after a series of subsequent reactions, they give rise to diazonium ions (RN_2^+), which then decompose to the final products (Scheme 3).

The diazonium salt formed from *N*-nitrosocysteine will decompose with release of nitrogen and formation of the corresponding organic compounds derived from the reaction of RN_2^+ with the various nucleophiles present in the reaction medium. The release of gas bubbles during the decomposition of *S*-nitrosocysteine is consistent with this reaction scheme. Likewise, this decomposition scheme of *S*-nitrosocysteine is in agreement with the absence of copper ion catalysis and the absence of nitrite ion and ammonia among the reaction products.

Further evidence of primary *N*-nitrosamine formation has been obtained by studying the stability of H_2NCH_2-CH_2S−NO under conditions analogous to that of *S*-nitrosocysteine. Among the reaction products, the alcohol $HOCH_2CH_2SH$ has been identified by ^{13}C NMR. The formation of this alcohol is consistent with the nitroso group transfer, generating a primary nitrosamine (ON−$NHCH_2CH_2SH$) which yields the diazonium salt ($^+N_2CH_2$-CH_2SH). This diazonium salt can be trapped by any nucleophile present in the reaction medium, and among the products it yields is $HOCH_2CH_2SH$.

The intermolecular nitroso group transfer is the basis of the widely studied transnitrosation reactions. Among the potential nitroso group donors, *O*-nitroso compounds (alkyl nitrites[27]), *N*-nitroso compounds (*N*-nitroso sulfonamides[28]), *S*-nitroso compounds (thionitrites[24,25,29]), among others, are well-known. There are many examples

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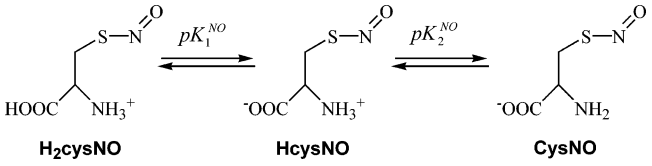
SCHEME 4



in the literature of reactions occurring with intramolecular nitroso group transfer. Among these intramolecular nitroso group transfers, the following are well-known: O−NO → N−NO migration, which can be observed in the nitrosation of amides and ureas,[30] nitrosation of amino acids[31] in acidic medium and nitrosation of hidroxylamines;[32] C−NO → N−NO migration, observed in the nitrosation of indoles[33] in acidic medium; and N−NO → C−NO migration, which can be observed in the Fischer−Hepp[34] rearrangement. Similarly, S−NO → N−NO rearrangements are common when studying the nitrosation of cysteine[35] in acidic medium, nitrosation of thioureas,[36] or the nitrosation of thioproline or thiomorpholine.[37] However, no evidence for S−NO → N−NO nitroso group transfers in neutral and basic media was found.

First-Order Term in Cysteine, k_1^{app}. The intramolecular nitroso group transfer is responsible for the first-order dependence of k_{obs} on cysteine concentration, whereas the intermolecular transfer is responsible for the quadratic dependence. *S*-Nitrosocysteine is in equilibrium with its corresponding conjugated acids and bases (Scheme 4).

Only the **CysNO** structure is able to transfer the nitroso group intramolecularly. In **H_2cysNO** and **HcysNO** structures, the protonated amino group is not nucleophilic enough for the rearrangement reaction of the NO group to occur.

A simple mechanism of intramolecular nitroso group transfer where only the *S*-nitrosocysteine molecule is involved (as shown in Scheme 2) would lead to a rate equation that would be zero-order in cysteine. This zero-order term ought to increase with increasing pH of the medium and reach a limiting value when all the *S*-nitrosocysteine is in the **CysNO** form. However, our experimental results show that there is a first-order term in cysteine, k_1^{app}, which exhibits a complex dependence on the pH of the medium. This first-order term arises from the fact that the intramolecular nitroso group transfer pathway proceeds with acid catalysis.

SCHEME 5

The reaction mechanism of *S*-nitroso compounds with nucleophiles is closely related to the nitrosation mechanism of thiols. Unlike nitrosation of alcohols,[15] nitrosation of thiols[38] takes place through a mechanism similar to that of nitrosation of amines. The attack of the nitrosating agent on the thiol occurs in a slow step, and the proton transfer occurs in a subsequent fast step (Scheme 5).

This nitrosation mechanism implies that denitrosation, NO group transfer to any other nucleophile, must occur with the assistance of acid catalysis. The existence of acid catalysis in the nitroso group transfer reaction will also be compatible with metal cation catalysis in the nitroso group transfer reaction from thiols to nucleophiles. This behavior is well-documented when studying the decomposition of nitrosothiols catalyzed by mercury(II) and silver salts.[39]

The decomposition reaction of *S*-nitrosocysteine occurs in buffer solutions in the presence of an excess of cysteine. Due to its acid−base equilibrium, cysteine can exist in five microscopic structures (Scheme 6) in function of the acidity of the reaction medium.

We must consider the possibility that four of the five structures of cysteine may act as acid catalysts in the nitroso group transfer reaction (Scheme 7).

Moreover, we must take into account the possibility that water and H^+ may also act as catalysts[40] given the wide range of pH values considered. This pattern of behavior allows us to obtain the following expression for k_{obs}, just considering that the total *S*-nitrosocysteine concentration will be the sum of the three structures shown in Scheme 4.

$$k_{obs} = \frac{K_1^{NO}K_2^{NO}}{K_1^{NO}K_2^{NO} + K_1^{NO}[H^+] + [H^+]^2}\left(k_{H_2O} + k_H[H^+] + \frac{(K_1K_Ak_{Hcys(-)} + K_1K_Bk_{CysH(-)})[H^+] + K_1k_{H2cys}[H^+]^2 + k_{H3cys(+)}[H^+]^3}{K_1K_AK_C + (K_1K_A + K_1K_B)[H^+] + K_1[H^+]^2 + [H^+]^3}[cysteine]_T\right) \quad (3)$$

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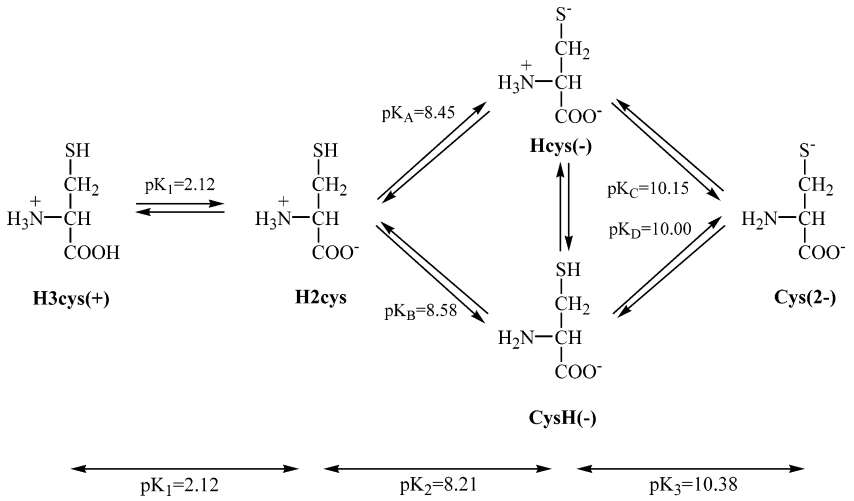
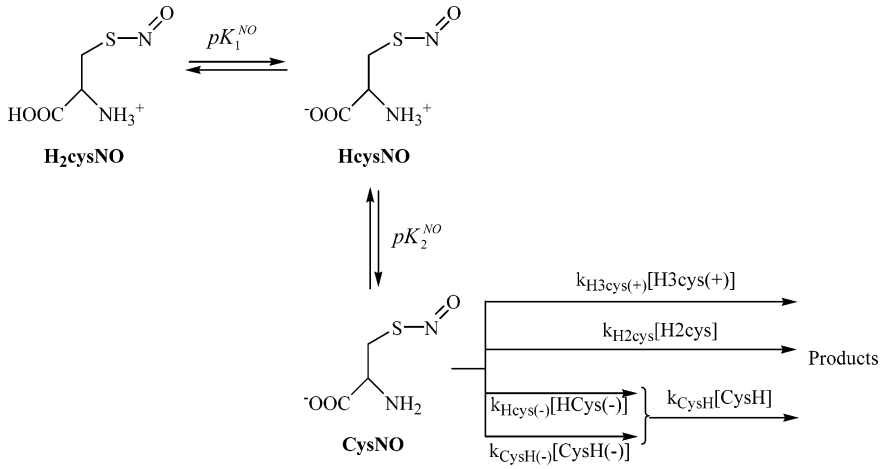
(40) When the decomposition reactions of *S*-nitrosocysteine were studied in the presence of a buffer different from cysteine itself, the existence of a nonzero intercept was observed in the linear dependence of k_{obs} on cysteine concentration. This intercept should correspond to the intramolecular nitroso group transfer reaction catalyzed by the acid form of the buffer.

SCHEME 6



SCHEME 7



From this equation we can obtain the following expression for k_1^{app}:

$$k_1^{app} = \frac{K_1^{NO}K_2^{NO}}{K_1^{NO}K_2^{NO} + K_1^{NO}[H^+] + [H^+]^2} \left(\frac{(K_1K_Ak_{Hcys(-)} + K_1K_Bk_{CysH(-)})[H^+] + K_1k_{H2cys}[H^+]^2 + k_{H3cys(+)}[H^+]^3}{K_1K_AK_C + (K_1K_A + K_1K_B)[H^+] + K_1[H^+]^2 + [H^+]^3} \right) \quad (4)$$

The rate constants $k_{Hcys(-)}$ and $k_{CysH(-)}$ cannot be obtained independently. We therefore turn to the macroscopic acidity constants, in such a way that the previous equation can be rewritten as:

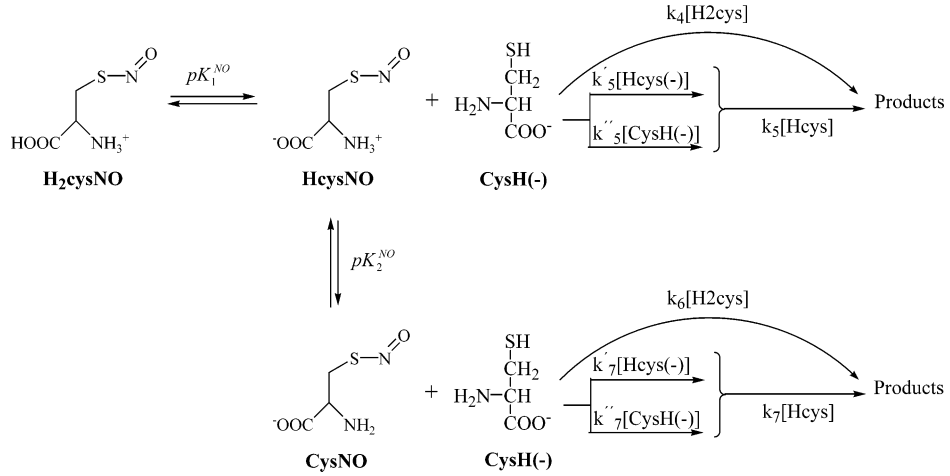
$$k_1^{app} = \frac{K_1^{NO}K_2^{NO}}{K_1^{NO}K_2^{NO} + K_1^{NO}[H^+] + [H^+]^2} \times \left(\frac{K_1K_2k_{Hcys}[H^+] + K_1k_{H2cys}[H^+]^2 + k_{H3cys(+)}[H^+]^3}{K_1K_2K_3 + K_1K_2[H^+] + K_1[H^+]^2 + [H^+]^3} \right) \quad (5)$$

where K_1, K_2, and K_3 are the macroscopic acidity constants of cysteine (see Scheme 6) and k_{Hcys} is the rate constant for acid catalysis by the macroscopic form, sum of **Hcys(−)** and **CysH(−)**. The previous equation can be simplified considering that the introduction of the nitroso group into a molecule causes an increase of acidity. Thus, it is expected that $K_1^{NO} \gg K_1$ and $K_2^{NO} \gg K_2$. Taking into account this increase in acidity when going from cysteine to *S*-nitrosocysteine and the values of the macroscopic acidity constants of cysteine (Scheme 6), we can rewrite the previous equation as:

$$k_1^{app} = \{K_1K_2K_2^{NO}k_{Hcys}[H^+] + K_1K_2^{NO}k_{H2cys}[H^+]^2 + K_2^{NO}k_{H3cys(+)}[H^+]^3\}/\{K_1K_2K_3K_2^{NO} + K_1K_2(K_2^{NO} + K_3)[H^+] + K_1(K_2^{NO} + K_2)[H^+]^2 + (K_2^{NO} + K_1)[H^+]^3 + [H^+]^4\} \quad (6)$$

This equation agrees perfectly with the observed kinetic behavior. The solid line shown in Figure 3 corresponds to the fit of experimental results to eq 6. From the fit of experimental results to eq 6, the values of $pK_1 = 3.4$, $pK_2 = 9.24$, $pK_3 = 10.67$, and $pK_2^{NO} = 6.12$ can be obtained. The values obtained for the pK_a of cysteine are close to the experimental results. The greatest deviations are observed for pK_1 and pK_2. In the case of pK_1, the reason lies in the fact that the acidity values used were not high enough for this term to have a great statistical weight. In addition, a certain disagreement is observed between the pK_2 value optimized from eq 6 and the literature value. The explanation lies mainly

SCHEME 8



in the existence of some degree of correlation between the pK_2 and pK_2^{NO} values when performing the mathematical fit. It must be emphasized that, as expected, the introduction of the nitroso group into the cysteine molecule brings about an increase in acidity. This increase in acidity confirms that $K_2^{NO} \gg K_2$.

Fitting eq 6 to the experimental results also allows us to obtain the values of the rate constants for the intramolecular nitroso group transfer catalyzed by cysteine. The values obtained, $k_{H3cys(+)} = 4.30\ M^{-1}\ s^{-1}$, $k_{H2cys} = 1.40 \times 10^{-2}\ M^{-1}\ s^{-1}$, and $k_{Hcys} = 4.90 \times 10^{-3}\ M^{-1}\ s^{-1}$, show that the reaction rate increases with increasing strength of the catalyst.

Second-Order Term in Cysteine, k_2^{app}. Figure 4 shows the results obtained from studying the influence of the acidity of the medium on k_2^{app}. As can be observed, this quadratic term is only observable in a pH range between 6.89 < pH < 12. The k_2^{app} value increases with increasing pH of the medium until it reaches a maximum value at pH ≈ 8 and then decreases with further increase in pH. This experimental behavior is assumed to be due to an intramolecular nitroso group transfer reaction catalyzed by a cysteine molecule.

Any of the three structures of *S*-nitrosocysteine—**H₂cysNO**, **HcysNO**, or **CysNO**—might transfer their nitroso group to an attacking nucleophile. Nevertheless, considering that the quadratic term, k_2^{app}, is observed only for values of pH > 6.9 and that the pK_1^{NO} value must be p$K_1^{NO} \leq 0$, only the **HcysNO** and **CysNO** structures are expected to be able to act as nitroso group donors. Only two of the five structures for cysteine shown in Scheme 6, **CysH(−)** and **Cys(2−)**, can act as nitroso group acceptors. These are the only microscopic structures of cysteine where the amino group is not protonated and therefore it can act as a nucleophile. The possibility of nucleophilic attack by the **Cys(2−)** structure is found to be negligible from the experimental results. If **Cys(2−)** were a reactive structure competing with **CysH(−)**, k_2^{app} would be expected to keep increasing for values of pH > 8, since a value of pK_3 = 10.38 corresponds to the **Cys(2−)** structure. Likewise, if the reactive structure were **Cys(2−)** it could not function as an acid catalyst, while the double behavior (both as a nucleophile and an acid catalyst) is possible for the **CysH(−)** structure. Therefore, we propose a reaction mechanism for the intermolecular nitroso group transfer as that shown in Scheme 8.

The **HcysNO** and **CysNO** structures of *S*-nitrosocysteine are able to act as nitroso group donors, whereas only the **CysH(−)** structure of cysteine can play the role of acceptor. We will consider as potential acid catalysts the structures of cysteine present in the acidity range between 6.9 < pH < 12, that is to say, **H2cys**, **Hcys(−)**, and **CysH(−)**. Similarly to what happened when studying the intramolecular nitroso group transfer mechanism, the reaction pathways of acid catalysis by **Hcys(−)** and **CysH(−)** are kinetically indistinguishable. Hence, we will use a rate constant corresponding to the pathway of acid catalysis by the macroscopic form, sum of **Hcys(−)** and **CysH(−)**.

The mechanism shown in Scheme 8 leads to the following expression for k_2^{app}:

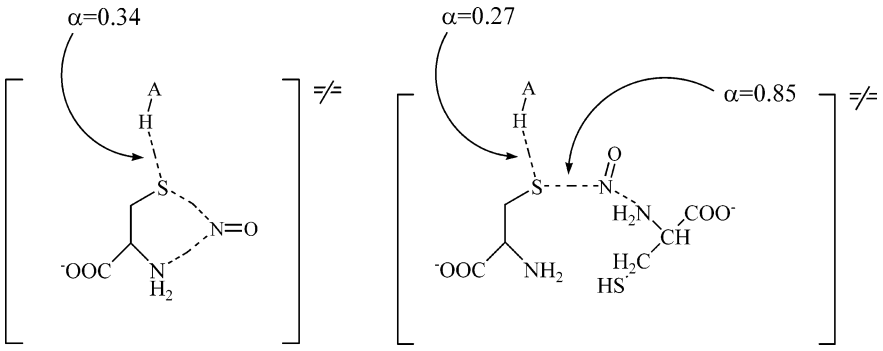
$$k_2^{app} = \frac{K_1}{K_2^{NO} + [H^+]} \times \frac{K_1K_AK_BK_2^{NO}k_7[H^+]^2 + (K_Ak_5 + K_2^{NO}k_6)K_1K_B[H^+]^3 + K_1K_Bk_4[H^+]^4}{\{K_1K_AK_C + (K_A + K_B)K_1[H^+] + K_1[H^+]^2 + [H^+]^3\}^2} \quad (7)$$

where the equilibrium and rate constants have the meanings ascribed to them by Schemes 4, 6, and 8. To obtain eq 7, we have considered that $K_2^{NO} \gg K_2$ just as when studying the influence of pH on k_1^{app}. In addition, it will be confirmed that $K_1 \gg [H^+]$ for the acidity range between 6.9 < pH < 12. Thus, the above equation can be rewritten as:

$$k_2^{app} = \frac{K_AK_B[H^+]^2}{\{K_AK_C + (K_A + K_B)[H^+] + [H^+]^2\}^2} \times \frac{k_7K_2^{NO} + \frac{(k_5K_A + k_6K_2^{NO})}{K_A}[H^+] + \frac{k_4}{K_A}[H^+]^2}{K_2^{NO} + [H^+]} \quad (8)$$

To fit eq 8 to the experimental results, we have kept constant the value for the microscopic acidity constant of cysteine, pK_B = 8.58. The values of pK_A = 8.23, pK_C =

SCHEME 9



11.27, and $pK_2^{NO} = 7.74$ can be obtained from the fit (solid line in Figure 4). The discrepancies between the experimental and optimized values of the acidity constants are a consequence of the narrow pH range where the quadratic dependence of the rate constant occurs, the cysteine concentration, and the great number of optimizable parameters. The values of $k_4 = 0.567\ M^{-2}\ s^{-1}$ and $k_7 = 2.1 \times 10^{-3}\ M^{-2}\ s^{-1}$ are also obtained. A value of $6.13 \times 10^{-9}\ M^{-1}\ s^{-1}$ is obtained for the term $(k_5K_A + k_6K_2^{NO})$.

The results obtained indicate that $k_4 > k_7$ as might be expected for a process of acid catalysis in terms of the strength of the acids involved. For the term $(k_5K_A + k_6K_2^{NO})$, we expect that $k_6 > k_5$ by virtue of the higher acidity of **H2cys** in comparison with **Hcys(−)** or **CysH(−)**. Furthermore, if we take into account that $K_A < K_2^{NO}$, we can conclude that $(k_5K_A + k_6K_2^{NO}) \approx k_6K_2^{NO}$. By using the value of $pK_2^{NO} = 6.12$ obtained previously, we calculated a value of $k_6 \approx 8.07 \times 10^{-3}\ M^{-2}\ s^{-1}$. This value is superior to the k_7 value obtained for the **Hcys**-catalyzed transnitrosation of **CysH(−)** from **CysNO**, and it is due to the greater acid strength of **H2cys** compared to **Hcys**. The k_6 value is also observed to be lower than that of k_4, although in both cases the acid catalyst is **H2cys**. The reason lies in the difference between the nitroso group donor agents: **HcysNO** is a better donor than **CysNO** since the leaving groups corresponding to the loss of the nitroso group would be the **Hcys(−)** and **Cys(2−)** structures (Scheme 6) to which it corresponds a difference in acidity of approximately two pK_a units.

Structure−Reactivity Correlations. From the values obtained for the rate constants of intramolecular nitroso group transfer, we can establish a Brønsted correlation (not shown) giving a value of $\alpha = (0.34 \pm 0.04)$. Similarly, a Brønsted correlation can be established for the rate constants of the intermolecular nitroso group transfer reaction. In this case, the correlation is more limited since only two values of the rate constants are provided. The value obtained, $\alpha = 0.27$, agrees well with that obtained for the intramolecular nitroso group transfer reaction. Given the fact that in both cases the nitroso group donor species is the same, **CysNO**, this result seems to indicate that the proton transfer in the transition state takes place to a small extent (Scheme 9).

The rate constants k_4 and k_6 correspond to intermolecular nitroso group transfer processes catalyzed by **H2cys** from different nitrosating agents. In the first case the nitrosating agent is **HcysNO** and its corresponding leaving group will be **Hcys(−)**, $(pK_2)_{lg} = 8.21$, while in the second case the leaving group will be **Cys(2−)**, $(pK_3)_{lg} = 10.38$. A Brønsted correlation can be constructed with the two available values of the rate constants, and then a slope, $\alpha_{lg} = 0.85$, is obtained. This value is indicative of the fact that the intermolecular nitroso group transfer reaction is proceeding through an asymmetric transition state in which the extent of S−NO bond cleavage is ahead of the proton transfer.

Conclusions

1. The reaction mechanism of the decomposition of *S*-nitrosocysteine has been studied under conditions of a large excess of cysteine. Analysis of the reaction products reveals the absence of nitrite ion and ammonia, indicating the existence of a new reaction pathway different from those considered traditionally.

2. The decomposition reaction has been found to proceed via one intramolecular and another intermolecular nitroso group transfer pathway. The first pathway constitutes the first example of intramolecular migration of the nitroso group (S−NO → N−NO) in neutral and basic medium. Kinetic analysis of the experimental results shows that both processes occur with acid catalysis by a cysteine molecule. An analysis of the experimental results allows us to establish a limited Brønsted correlation and to propose a structure for the transition state of the reaction where the extent of S···NO bond cleavage is greater than that of AH···S bond formation.

3. The existence of a new decomposition pathway for *S*-nitrosocysteine in which NO is not produced has a significant biological impact since nitrosothiols have usually been considered as potential NO donors in vivo.

Acknowledgment. Financial support from the Xunta de Galicia (PGIDT03-PXIC20905PN and PGIDIT04-TMT209003PR) and Ministerio de Ciencia y Tecnología (Project BQU2002-01184 and HP03-34) is gratefully acknowledged. C.A. thanks the University of Santiago for a postdoctoral fellowship.

JO050811R