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Biological Thiols and Carbon Disulfide: The Formation and Decay of Trithiocarbonates under Physiologically Relevant Conditions

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Supporting Information

ABSTRACT: Carbon disulfide is an environmental toxin, but there are suggestions in the literature that it may also have regulatory and/or therapeutic roles in mammalian physiology. Thiols or thiolates would be likely biological targets for an electrophile, such as CS₂, and in this context, the present study examines the dynamics of CS₂ reactions with various thiols (RSH) in physiologically relevant near-neutral aqueous media to form the respective trithiocarbonate anions (TTC−), also known as "thioxanthate anions". The rates of TTC− formation are markedly pH-dependent, indicating that the reactive form of RSH is the conjugate base RS−. The rates of the reverse reaction, that is, decay of TTC− anions to release CS₂, is pH-independent, with rates roughly antiparallel to the basicities of the RS− conjugate base. These observations indicate that the rate-limiting step of decay is simple CS₂ dissociation from RS−, and according to microscopic reversibility, the transition state of TTC− formation would be simple addition of the RS− nucleophile to the CS₂ electrophile. At pH 7.4 and 37 °C, cysteine and glutathione react with CS₂ at a similar rate but the trithiocarbonate product undergoes a slow cyclization to give 2-thiothiazolidine-4-carboxylic acid. The potential biological relevance of these observations is briefly discussed.

INTRODUCTION

This laboratory has recently compiled literature information on the known and suggested physiological properties of carbon disulfide (CS₂) and identified certain analogies to the small-molecule bioregulators (SMBs) nitric oxide, carbon monoxide, and hydrogen sulfide (sometimes called "gasotransmitters"). The common properties of these SMBs include partial solubility in aqueous and lipid systems, the ability to diffuse readily in physiological structures, and known toxicity at higher concentrations. Similarly, CS₂ is a nonpolar, readily diffusible molecule considered to be an environmental toxin. In addition, there are indications that CS₂ is formed endogenously or in the associated gut microbiome of mammals. Biological sulfhydryls (R−SH) would be likely targets, and the dynamics of the "on" and "off" reactions of this electrophile with such nucleophiles should be crucial to any physiological roles. Thus, the present study is focused on exploring the reactivity of CS₂ with thiols, such as cysteine (CysSH) and glutathione (GSH), as well as with several model thiols to form trithiocarbonate anions (TTC−, also known as "thioxanthate anions") in near-neutral aqueous media (eq 1). From the biomedical perspective, the trithiocarbonate anion (PhCH₂SCS₂−) has been studied as an inhibitor to carbonic anhydrases and as a possible therapeutic in glaucoma treatment. However, to our knowledge, the dynamics of the formation and decay of TTC salts under physiologically relevant conditions have not been previously reported.

\[
\text{CS}_2 + \text{RSH} \rightleftharpoons \text{R} \text{S}^- + \text{H}^+ + \text{TTC}^- \tag{1}
\]

Elucidating prospective biological roles of carbon disulfide will depend on having vehicles for controlled CS₂ release under experimental biological conditions. Certain TTCs are unstable toward the slow release of carbon disulfide, and such reactivity may be relevant to the biological activity of CS₂, as well as a desirable property for CS₂ delivery. In this context, we describe the kinetics of CS₂ dissociation from several prepared TTC− salts in aqueous solution. The latter studies complement earlier investigations of CS₂ generation by photosensitized oxidation of 1,1-dithiooxalate and by the thermal decay of dithiocarbamate anions. The CS₂ release rates from the latter precursors vary considerably, thereby providing a wide range of activities for physiological experiments. The TTC derivatives of CysSH and GSH also undergo a slow cyclization reaction to give 2-thiothiazolidine-4-carboxylic acid (TTCA), a product that, when found in the urine, is considered diagnostic of exposure...
to carbon disulfide\textsuperscript{9,10}. Notably, this cyclization also releases an equivalent of hydrogen sulfide.

\textbf{RESULTS AND DISCUSSION}

\textbf{Thioisocyanate Decay.} As noted above, we have recently described the kinetics for a set of dithiocarbamate salts that decay by releasing CS\textsubscript{2} with lifetimes ranging from seconds to days in near-neutral, aerobic aqueous media at 37 °C\textsuperscript{8}. In this section, we describe analogous decays of several RSC\textsubscript{n}CS\textsubscript{2}− anions under similar conditions.

The TTC\textsuperscript{−} salts used here were prepared by the reaction of the corresponding thiol precursor with CS\textsubscript{2} in strongly alkaline solution. The electronic spectrum of each displays intense absorption bands at approximately 310 and 330 nm with extinction coefficient of \( \sim 8 \times 10^{4} \) M\textsuperscript{−1} cm\textsuperscript{−1} (e.g., Figure 1) that we assign to \( \pi \rightarrow \pi^{*} \) transitions largely localized on the –SCS\textsubscript{n}− functional group. Time-dependent density functional theory calculations (Supporting Information (SI) Figure S1) support this assignment. Similar but somewhat higher-energy absorptions are seen in the spectra of analogous dithiocarbamate (R\textsubscript{n}NCS\textsubscript{2}−) and xanthate (ROCS\textsubscript{2}−) anions\textsuperscript{8,11}. Decays of the TTC anions in aqueous media are readily followed by the temporal decreases of these two UV bands (Figure 1). These spectral changes were accompanied by the release of at least 90% of the CS\textsubscript{2} predicted, for example, by the stoichiometry of eq 2, using the CS\textsubscript{2} analysis method described in detail elsewhere\textsuperscript{7,8} and briefly in the Experimental Section.

\[ \text{PTTC}^- \quad \text{eq 2} \]

As discussed below, the decays of these TTC\textsuperscript{−} anions are reversible; therefore, it is necessary to take the back-reaction into account as indicated in eq 3 when evaluating the kinetics of the temporal spectral changes. These reactions were conducted in buffered solutions at specific pH values, and the effects of pH, buffer, and other factors are incorporated into the apparent rate constants \( k_{\text{off}} \) and \( k_{\text{on}} \) for the forward reaction and back-reaction. Although [CS\textsubscript{2}] is not constant during an individual experiment, the temporal spectral data can be fit numerically using a nonlinear least-squares regression program (see Experimental Section and SI Figure S2); however, the \( k_{\text{on}} \) values so obtained are inherently less accurate than are the corresponding \( k_{\text{off}} \) values.

\[ \frac{d[\text{TTC}^-]}{dt} = k_{\text{off}}[\text{TTC}^-] - k_{\text{on}}[\text{CS}_2][\text{RSH}] \quad \text{eq 3} \]

Figure 1 illustrates temporal spectral changes observed when a sample of the sodium salt of 3-trithiocarbonatopropionate (Na\textsubscript{2}[PTTC]) undergoes decay in pH 7.4 aqueous solution at 37 °C (eq 2). Table 1 summarizes the rate constants \( k_{\text{off}} \) and \( k_{\text{on}} \)

calculated as described. There was no significant effect of pH on \( k_{\text{off}} \) over the range 6.5–7.8; thus, the decay of PTTC\textsuperscript{−} is not catalyzed by acid, an observation that is in direct contrast to the decay under analogous conditions of the dithiocarbamate ions. The \( k_{\text{on}} \) values for the reverse reaction appear to increase with pH, as would be expected, if this step involves the reaction of the electrophile CS\textsubscript{2} with the thiolate group of the product. This type of reactivity is discussed in greater detail below. No significant effects were seen for increasing ionic strength from 0.154 to 0.308 M or buffer concentration from 50 to 100 mM.

A linear Eyring plot of the \( k_{\text{off}} \) values determined for the decay of PTTC\textsuperscript{−} in pH 7.4 aqueous buffer solution over the temperature range of 5–55 °C (SI Figure S3) gave the activation parameter values \( \Delta H^\dagger = 70.3 \pm 0.7 \) kJ mol\textsuperscript{−1} and \( \Delta S^\dagger = -69 \pm 2 \) J K\textsuperscript{−1} mol\textsuperscript{−1}.

\[ \text{MeO}_2\text{C(CH}_3)_2\text{S}^- + \text{H}^+ \xrightarrow{\text{on}} \text{CS}_2 + \text{MeO}_2\text{C(CH}_3)_2\text{SH} \quad \text{eq 4} \]

MPTTC\textsuperscript{−}

N-Acetylcyisteine trithiocarbonato anion (NacTTC\textsuperscript{−}, eq 5) behaved similarly but gave larger values for both \( k_{\text{off}} \) (0.0118 ± 0.004 s\textsuperscript{−1}) and \( k_{\text{on}} \) (0.7 ± 0.2 M\textsuperscript{−1} s\textsuperscript{−1}) in pH 7.4 aqueous phosphate buffer solution (100 mM) at 37 °C. An Eyring plot of \( k_{\text{off}} \) values measured over the temperature range of 5–55 °C (SI Figure S3) gave the activation parameter values \( \Delta H^\dagger = 66.8 \pm 0.9 \) kJ mol\textsuperscript{−1} and \( \Delta S^\dagger = -67 \pm 3 \) J K\textsuperscript{−1} mol\textsuperscript{−1} quite similar to those determined for the decay of PTTC\textsuperscript{−}.
the conjugate thiolate anion, $RS^-$, consistent with the rate-limiting step dissociation of $CS_2$ from solution pH over the range of $6.5$–$10.1$ (Table 2). In contrast, the $k_{on}$ value calculated was markedly larger at the highest pH, and this was reflected by the reaction not going to completion but reaching an equilibrium or steady state (Figure 2).

**Table 2.** $k_{off}$ and $k_{on}$ Values Determined for the Decay of BnTTC$^-$ in Aqueous Phosphate Buffer Solution at 37 °C

<table>
<thead>
<tr>
<th>pH°</th>
<th>$k_{off}$ (in $10^{-3}$ s$^{-1}$)</th>
<th>$k_{on}$ (M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>8.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>8.5 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>7.8</td>
<td>8.5 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>10.1</td>
<td>7.3 ± 0.1</td>
<td>58 ± 1</td>
</tr>
</tbody>
</table>

"100 mM phosphate buffer and $\mu = 0.18$–$0.29$ M."

The absence of a pH effect on the $k_{off}$ values in 37 °C aqueous media for the decays of PTTC$^-$ and BnTTC$^-$ is consistent with the rate-limiting step dissociation of $CS_2$ from the conjugate thiolate anion, $RS^-$, followed by protonation of the latter, as illustrated in Scheme 1. In accord with this sequence where the first step is rate limiting, that is $k_{off} = k_i$, the reactivity order NacTTC$^-$ > BnTTC$^-$ > PTTC$^-$ > MPTTC at pH 7.4 is roughly antiparallel to the increasing basicity of the thiolate anion $RS^-$ as reflected in $pK_a$ values of the respective thiols (RSH) (SI Table S1).12–16

In other words, the more basic thiolate ions are slower to dissociate from the $CS_2$ electrophile. However, one might expect a positive value of $\Delta S^\ddagger$ for unimolecular dissociation of the $RSH$–$CS_2$ bond illustrated in the first step of Scheme 1, but that was not the case for PTTC$^-$, MPTTC$^-$, or NacTTC$^-$. One possible explanation for the observed negative $\Delta S^\ddagger$ values would be a pathway involving concerted protonation of the exiting thiolate group by a general acid (solvent or buffer conjugate acid). However, no general acid catalysis was observed and protonation by $H_2O$ itself would generate hydroxide ion, which would be unfavorable. Thus, a more likely explanation for a negative entropy of activation draws from solvent reorganization as the negative charge delocalized over the $−CS_2^−$ functional group becomes localized on the thiolate ion at the transition state (Scheme 2).

**Scheme 1.** Proposed Sequence of Steps Leading to TTC$^-$ Decay in Aqueous Media

$$
RS-\text{CS}_2^- \overset{k_i}{\rightarrow} RS^- + \text{CS}_2
$$

**Scheme 2.** Solvation Reorganization upon $CS_2$ Dissociation from a TTC$^-$ Anion

**Figure 2.** Effect of pH on the decay kinetics of BnTTC$^-$ at 37 °C and in 0.1 M phosphate buffer, except for pH 10.1, which is in 0.1 M carbonate buffer (all experiments done in duplicate).

**Figure 3.** illustrates the temporal spectrum changes that occur rapidly after stopped-flow mixing of a solution containing excess cysteine (CysSH) with a second solution containing $CS_2$ in pH 7.4 aqueous buffer at 37 °C. Very similar spectral changes were shown to result from the reactions of $CS_2$ with (respectively) N-acetylcysteine (NacSH), glutathione (GSH, SI Figure S6), and cysteine methyl ester (MecSH, SI Figure S7). In each case, the appearance of the spectrum characteristic of a TTC$^-$ anion (eq 7) followed an exponential rise as seen in the figure inset.

**Formation of Trithiocarbonates.** Physiologically, thiols are likely targets in the action of $CS_2$ either as a toxin or in potential bioregulatory or therapeutic roles.1 For example, modification of a key protein thiol by the formation of trithiocarbonate would be expected to have profound effects on that protein’s activity. The “off” reaction noted above is, of course, the formation of the TTC$^-$ adduct from the parent thiol plus $CS_2$. The goal in this section is to examine the dynamics of trithiocarbonate formation with cysteine and several cysteine derivatives, including glutathione, in greater detail.

**Scheme 1. Proposed Sequence of Steps Leading to TTC$^-$ Decay in Aqueous Media**

$$
RS-\text{CS}_2^- \overset{k_i}{\rightarrow} RS^- + \text{CS}_2
$$

$$
RS^- + \text{H}^+ \overset{k_2}{\rightarrow} RSH
$$

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6537
relaxing to equilibrium and CS₂ is the limiting reactant, the relationship described by eq 8 holds true:\(^1\)

\[ k_{\text{obs}} = k_{\text{on}} + k_{\text{off}}[\text{RSH}] \]  

(8)

Thus, when RSH = CysSH, a plot of \( k_{\text{obs}} \) versus [CysSH] should be linear with a slope equal to \( k_{\text{on}} \) and an intercept equal to \( k_{\text{off}} \). The inset of Figure 4 is such a plot for the reaction of CS₂ with excess CysSH in 37 °C, pH 7.4 buffered aqueous solutions from which the values \( k_{\text{on}} = 2.9 \pm 0.1 \text{ M}^{-1} \text{s}^{-1} \) and \( k_{\text{off}} = 0.103 \pm 0.002 \text{ s}^{-1} \) were determined. Plots similar to Figure 4 were generated for the reactions of glutathione (GSH), cysteine methyl ester (MecSH), and N-acetylcysteine (NacSH) (SI Figures S6–S8), and the \( k_{\text{on}} \) and \( k_{\text{off}} \) values so determined are summarized in Table 3. Notably, the values of \( k_{\text{on}} \) and \( k_{\text{off}} \) determined in 37 °C, pH 7.4 solution for NacSH (0.606 ± 0.008 M⁻¹ s⁻¹ and 0.0138 ± 0.001 s⁻¹, respectively) can be compared to those (0.7 ± 0.2 M⁻¹ s⁻¹ and 0.018 ± 0.004 s⁻¹) obtained above by following the decay of NacTTC under similar conditions. Given the different procedures and apparatus used in these two experiments, the agreement is quite good.

As noted above for several other TTC anions, the \( k_{\text{off}} \) values reported in Table 3 for the cysteine derivatives decrease as the basicity of the RS⁻ increases owing presumably to the higher RS⁻–CS₂⁻ bond strength through this series. The values of \( k_{\text{on}} \) at pH 7.4 show a similar decrease (hence, the ratio \( k_{\text{on}}/k_{\text{off}} \) varies only modestly over this series). The behavior of \( k_{\text{on}} \) would be consistent with the mechanism suggested by the microscopic reverse of Scheme 1. If \( k_{\text{on}} = k_{f} \), then \( k_{\text{on}} = k_{f}(H^+) \), where \( f(H^+) = K_f/(K_a + [H^+]) \). As the acidity of RSH (\( K_a \)) increases, more of the conjugate base thiolate RS⁻ is available at pH 7.4. Thus, one would expect \( k_{\text{on}} \) to increase. However, the nucleophility of RS⁻ is likewise also decreasing over this series, so one might expect the rate constant \( k_f \) for the nucleophilic attack of RS⁻ on CS₂ to correspondingly decrease in going from NacS⁻ to MeCysS⁻, the two trends therefore countering each other. This may help account for a reactivity difference on only an order of magnitude between NacSH and MeCysSH at pH 7.4 despite the much greater difference in \( K_a \) values.

SI Figure S9 displays Eyring plots of the \( k_{\text{on}} \) and \( k_{\text{off}} \) values determined in a similar manner for the reaction of CysSH with CS₂ in pH 7.4 buffered aqueous solution over the temperature range of 25–45 °C. The apparent activation parameters for \( k_{\text{on}} \) are \( \Delta H^\ddagger = +843.4 \text{ kJ mol}^{-1} \) and \( \Delta S^\ddagger = +37.4 \text{ J K}^{-1} \text{ mol}^{-1} \), and those for \( k_{\text{off}} \) are \( \Delta H_{\text{off}}^\ddagger = +75.1 \text{ kJ mol}^{-1} \) and \( \Delta S_{\text{off}}^\ddagger = -21.7 \text{ J K}^{-1} \text{ mol}^{-1} \). Notably, \( \Delta H^\ddagger \) for \( k_{\text{off}} \) in this case are quite similar to that recorded above at pH 7.4 for the decays of PTTC⁻, MPTTC⁻, and NacTTC⁻, but because \( \Delta S^\ddagger \) remains negative in this case, it is less so than for the other three (SI Table S2). According to the proposed mechanism described in Scheme 1, \( k_{\text{on}} = k_f \), so the apparent \( \Delta H^\ddagger \) and \( \Delta S^\ddagger \) for this step equal \( \Delta H_f^\ddagger \) and \( \Delta S_f^\ddagger \), respectively. The relationship is more complex for \( k_{\text{on}} \) because it equals \( k_f(H^+) \). If one were to make the rough approximation that, at pH 7.4, \( [H^+] \approx K_a \), then \( k_{\text{on}} \approx k_fK_a[H^+] \) and then \( \Delta H_{\text{on}}^\ddagger = \Delta H_f^\ddagger + \Delta H_0^\ddagger \) and \( \Delta S_{\text{on}}^\ddagger = \Delta S_f^\ddagger + \Delta S_0^\ddagger \). The values of \( \Delta H_f^\ddagger \) and \( \Delta S_f^\ddagger \) can be calculated as +30.9 kJ/mol and −52 J K⁻¹ mol⁻¹, respectively, from the pH dependence of the \( K_a \) of cysteine (SI Figure S10).\(^1\) Thus, on the basis of this model, \( \Delta H_f^\ddagger \approx 53 \text{ kJ mol}^{-1} \) and \( \Delta S_f^\ddagger \approx +89 \text{ J K}^{-1} \text{ mol}^{-1} \) for the reaction of CysSH with CS₂.

Table 4 reports the results of analogous stopped-flow kinetics studies of the reactions of CysSH and GSH with CS₂ to form

![Figure 4](image-url)
Table 3. Values of \( k_{\text{on}} \) and \( k_{\text{off}} \) Determined Using a Stopped-Flow Kinetics Spectrophotometer for the Reactions of CysSH, NacSH, MecSH, and GSH with CS\(_2\) in 37 °C, pH 7.4 Buffered Aqueous Solution\(^a\)

<table>
<thead>
<tr>
<th>RSH</th>
<th>( p_{K_a} ) (SH)</th>
<th>( p_{K_a} (\text{NH}_3^+) )</th>
<th>( k_{\text{on}} ) (M(^{-1}) s(^{-1}))</th>
<th>( k_{\text{off}} ) (s(^{-1}))</th>
<th>( k_{\text{on}}/k_{\text{off}} ) (M(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>MecSH</td>
<td>6.56(^a)</td>
<td>8.99(^b)</td>
<td>4.3 ± 0.1</td>
<td>0.116 ± 0.002</td>
<td>37</td>
</tr>
<tr>
<td>CysSH</td>
<td>8.33(^a)</td>
<td>10.78</td>
<td>2.9 ± 0.1</td>
<td>0.103 ± 0.002</td>
<td>28</td>
</tr>
<tr>
<td>GSH</td>
<td>8.96(^a)</td>
<td>9.12(^b)</td>
<td>2.5 ± 0.1</td>
<td>0.036 ± 0.001</td>
<td>69</td>
</tr>
<tr>
<td>NacSH</td>
<td>9.52(^a)</td>
<td></td>
<td>0.606 ± 0.004</td>
<td>0.0138 ± 0.001</td>
<td>44</td>
</tr>
</tbody>
</table>

\(^a\) [CS\(_2\)] = 0.5 mM, 100 mM phosphate buffer solution, \( \mu = 0.308 \) M. \(^b\) Ref 18. Ref 19.

Table 4. Rate Constants Measured for Reaction between CS\(_2\) (0.1 mM) and CysSH (1–8 mM) or GSH (1–8 mM) as a Function of pH at 37 °C in Buffered Aqueous Solution

<table>
<thead>
<tr>
<th></th>
<th>CysSH</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>( k_{\text{on}} ) (M(^{-1}) s(^{-1}))</td>
<td>( k_{\text{off}} ) (s(^{-1}))</td>
</tr>
<tr>
<td>7.4</td>
<td>2.9</td>
<td>0.103</td>
</tr>
<tr>
<td>7.6</td>
<td>6.4</td>
<td>0.099</td>
</tr>
<tr>
<td>8.0</td>
<td>11.7</td>
<td>0.086</td>
</tr>
<tr>
<td>8.4</td>
<td>14.9</td>
<td>0.075</td>
</tr>
<tr>
<td>8.8</td>
<td>18.4</td>
<td>0.054</td>
</tr>
<tr>
<td>9.2</td>
<td>22.0</td>
<td>0.0!3</td>
</tr>
<tr>
<td>9.6</td>
<td>26.0</td>
<td>0.023</td>
</tr>
</tbody>
</table>

CysTTC\(^-\) and GTTC\(^-\) (eqs 7 and 9, respectively) at different pH values over the range of 7.4–9.6. The \( k_{\text{on}} \) values were determined for a range of different initial CysSH concentrations at each pH, and linear plots of \( k_{\text{on}} \) versus [CysSH] similar to those shown in Figure 4 and SI Figures S6–S8 gave the pH-dependent values of \( k_{\text{on}} \) and \( k_{\text{off}} \). Both systems show dramatic increases in \( k_{\text{on}} \) at higher pH as anticipated from the simple mechanism proposed in Scheme 1. Consistent with the experiments for PTTTC\(^-\) and BnTTC\(^-\) reported in Tables 1 and 2, the \( k_{\text{on}} \) values for GTTC\(^-\) are pH-independent; however, this was not the case for CysTTC\(^-\), for which \( k_{\text{on}} \) decreases by about a factor of four from pH 7.4 to 9.6. We attribute this difference to the relative proximity of the protonated amine group (\( \text{NH}_3^+ \)) to the trithiocarbonate functionality in CysTTC\(^-\). The resulting change in the inductive effect upon deprotonation of this group at higher pH should enhance the basicity of the thiolate, thus leading to slower dissociation of CS\(_2\). Although GTTC\(^-\) has a similar amine, it is positioned further from the TTC functionality and its deprotonation would have a much smaller impact.

\[ \text{CysTTC}^- + \text{CS}_2 \rightarrow \text{CysTTC}^\text{SC}^- \]

\[ \text{CysTTC}^- + \text{CS}_2 \rightarrow \text{CysTTC}^\text{SC}^- \]

Subsequent Reactions of CysTTC\(^-\) and GTTC\(^-.\)

Although the reaction of CS\(_2\) with cysteine initially showed the rapid appearance of new absorption bands at ∼295 and 332 nm consistent with the formation of the trithiocarbonate CysTTC\(^-\) (eq 7), a much slower subsequent reaction was evidenced by further spectral changes (Figure 5) involving the disappearance of the bands at ∼295 and 332 nm and the appearance of a new band at 270 nm. The latter band can be assigned to the cyclized compound thiazolidine-2-thione-carboxylate (TTCA, eq 10), which has been identified\(^{20,21}\) as a urinary excretion product from humans who have been exposed to CS\(_2\). The temporal decay at 332 nm and rise at 270 nm could be fit to exponential functions to give the respective first-order rate constants 5.2 × 10\(^{-5}\) and 4.9 × 10\(^{-3}\) s\(^{-1}\) in pH 7.4 aq phosphate buffer at 37 °C (SI Figure S11). However, inspection of the spectral changes shows the absence of isosbestic points, as well as an absorbance increase followed by a decrease of a shoulder at ∼230 nm, which are clear indications that a transient intermediate species is formed. When the reaction was run with a very large excess of CysSH ([CS\(_2\)] = 0.2 mM; [CysSH] = 10 mM), the \( k_{\text{on}} \) value measured was nearly the same (5.8 × 10\(^{-3}\) s\(^{-1}\)). Thus, it appears that this slow cyclization process is unimolecular and does not involve the reaction of free CysSH.

Figure 5. Spectral changes upon mixing CS\(_2\) (1 mM) with CysSH (1 mM) in pH 7.4 aq phosphate buffer (100 mM) at 37 °C in a sealed vial indicating the slow transformation of CysTTC\(^-\) (red spectrum) to TTCA (blue spectrum). Total time = 17 h. Spectra recorded at 600 s intervals.

The reaction of cysteine (10 mM) and CS\(_2\) (10 mM) was further studied in a buffered phosphate deuterium oxide solution (prepared with anhydrous Na\(_3\)PO\(_4\) plus D\(_2\)O, pD 7.5) to characterize more thoroughly the formation of TTCA. DCI solution (35 wt % in D\(_2\)O) was used to correct the pD. The
reaction was run for 24 h at 37 °C. A small aliquot of the product solution was used to check the electronic spectrum, which showed the presence of a single and intense absorption band at 271 nm (SI Figure S12), indicating the formation of the cyclic compound because unreacted cysteine and oxidized cysteine products, such as cystine, have no strong absorption bands in this wavelength range. The $^1$H NMR spectrum of the product solution (SI Figure S13) showed not only resonances belonging to unreacted cysteine but also two groups of proton resonances at 3.67–3.63 ppm (S–CH$_2$ doublet of doublets) and 3.92–3.89 (CH, broad doublet of doublets) attributed by van Doorn et al. to the hydrogens of TTC.$^{22}$

GTTC$^-$ formed by the relatively rapid reaction of GSH with CS$_2$ also goes through a very slow transformation to a new species reported to be TTCA (SI Figure S14). However, similar spectral changes/secondary reaction was not seen with 3-trithiocarbonatopropionate (PTTC$^-$) but was observed with 3-trithiocarbonatopropylamine and thus an amine functionality appears to be required. This is not simply the transfer of CS$_2$ from the thiolate to the amine to form a dithiocarbamate (DTC$^-$) analogue because the spectrum formed by the reaction of aqueous base and glycine displays bands at 253 and 284 nm (data not shown) typical of DTC anions and very different from the characteristic of TTCA. The mechanism(s) of these transformations are the subject of continuing investigation.

## SUMMARY

We have described the kinetics of the formation and decay of trithiocarbonate derivatives formed by the reactions of carbon disulfide and various thiols (RSH) under physiologically relevant conditions (near-neutral pH, 37 °C). Rates of TTC$^-$ formation are strongly dependent on solution pH, indicating that the rate-limiting step involves the reaction of the thiolate anion RS$^-$ with the electrophilic CS$_2$ substrate. Rates of TTC$^-$ decay are pH-independent, consistent with microscopic reversibility. The relative decay rates have a reverse correlation to the basicity of the RS$^-$ anions as expected if simple dissociation of CS$_2$ is rate determining, as illustrated by the qualitative reaction coordinate diagram shown in Figure 6. At pH 7.4, glutathione and cysteine react with CS$_2$ at similar rates, and in both cases, the resulting TTC$^-$ anions undergo slow cyclization reactions to a cyclized species identified previously as 2-thiothiazolidine-4-carboxylic acid. As noted above, the latter has been found to be urinary excretion products from as 2-thiothiazolidine-4-carboxylic acid. As noted above, the latter has been used to check the electronic spectrum, which showed the presence of a single and intense absorption band at 271 nm (SI Figure S12), indicating the formation of the cyclic compound because unreacted cysteine and oxidized cysteine products, such as cystine, have no strong absorption bands in this wavelength range. The $^1$H NMR spectrum of the product solution (SI Figure S13) showed not only resonances belonging to unreacted cysteine but also two groups of proton resonances at 3.67–3.63 ppm (S–CH$_2$ doublet of doublets) and 3.92–3.89 (CH, broad doublet of doublets) attributed by van Doorn et al. to the hydrogens of TTC.$^{22}$

![Figure 6](https://example.com/figure6.png)

Figure 6. Qualitative reaction coordinate diagram for the formation ($k_{on}$) and decay ($k_{off}$) of trithiocarbonate anions (RSCS$_2^-$) from the respective thiols plus carbon disulfide. According to Scheme 1, TTC$^-$ decay is independent of pH ($k_{off} = k_{on}$), whereas the forward reaction is strongly pH-dependent owing to the RSH $\rightleftharpoons$ RS$^-$ + H$^+$ equilibrium.

reported for metal–sulfur bonds$^{25,26}$ as well as with alkoxide,$^{27}$ amine$^{28,29}$ and (even) phosphate$^{30}$ ligands. Given the relatively high cytosolic concentrations of GSH$^{31}$ and plasma-based protein sulphhydryls,$^{32}$ the reversible formation of unstabilized TTCs may play a role in CS$_2$ transport.

## EXPERIMENTAL SECTION

### Materials

Except where otherwise noted, all materials were of analytical or reagent grade and were used without further purification. N-Acetyl-$\beta$-cysteine (≥99%, TLC), 3-mercaptopropionic acid (≥99%), i-cysteine (97%), glycine (≥98.5%), and reduced i-glutathione (98%) were purchased from Sigma-Aldrich. Carbon disulfide (ACS Reagent Grade, ≥99.9%), benzyl mercaptan (Alfa Aesar, 99%), potassium hydroxide, sodium hydroxide, and mono- and dibasic sodium phosphate and sodium chloride used to prepare buffers were purchased from Fisher Scientific.

### Synthesis of TTCs. Sodium 3-(Trithiocarbonato)-propionate (Na$_3$PTTC$^-$).

This species had been generated as an intermediate in the preparation of a corresponding trithiocarbonate ester.$^{33}$ A round-bottom flask was charged with pentane and CS$_2$ (30 mL each) plus a stir bar and then was cooled to approximately 0–5 °C with an ice bath and purged with inert gas. During rapid stirring, finely ground NaOH (3.09 g, 77.4 mmol) was added to the flask under inert gas. Sufficient water was added to solubilize the hydroxide (1–3 mL), and to the resulting base solution, 3-mercaptopropionic acid (4.11 g, 38.7 mmol) in diethyl ether (10 mL) was added dropwise over the course of a few minutes. A deep yellow powder immediately began to precipitate. The reaction was allowed to warm to room temperature, and stirring was continued for 6–12 h. The solid was collected via filtration and rinsed with cold Et$_2$O. The yield was 3.68 g (37%). The product proved to be soluble only in methanol or water; however, attempts to recrystallize from these solvents resulted in decomposition. The optical spectrum of the initial product in pH 7.4 water displayed bands at $\lambda_{max}$ = 303 and 332 nm with an extinction coefficient at 332 nm (8650 M$^{-1}$ cm$^{-1}$) close to
those previously observed for alkyl TTCs. The infrared spectrum and CHN analysis are consistent with a hydrated sample. The solid is rather odorous. The compounds were kept in a freezer for long-term storage.

Optical spectrum: \( \text{Ethanol} \) resulted again in a golden oil. This product was stored resulting solution stirred vigorously.

Vacuum removal of the mmol) was added. Dimethylformamide (2 mM) was added dropwise, resulting in rapid separation of an insoluble, viscous, translucent, golden oil. This oil was separated from the solvent by decanting and was dried in vacuo for several days at elevated temperature (\( \sim 40^\circ C \)). The oil was then dissolved in ethanol, heated gently, and the resulting solution stirred vigorously. Vacuum removal of the ethanol resulted again in a golden oil. This product was stored and stored in a freezer. The yield was 4.58 g (54%).

Triethylammonium (N-Acetylcysteine)trithiocarbonate ([HNEt3][NacTTC]). To an ice-cold (0 to 5 °C) flask containing diethyl ether (30 mL) and triethylamine (3.72 g, 36.8 mmol) under inert atmosphere, a 3 g portion of N-acetylcysteine (18.4 mmol) was added. Dimethylformamide (2–3 drops) was added to solubilize the N-acetylcysteine, after which C\(_2\)S\(_2\) (1.90 g, 25 mM) was added dropwise, resulting in rapid separation of an insoluble, viscous, translucent, golden oil. This oil was separated from the solvent by decanting and was dried in vacuo for several days at elevated temperature (\( \sim 40^\circ C \)). The oil was then dissolved in ethanol, heated gently, and the resulting solution stirred vigorously. Vacuum removal of the ethanol resulted again in a golden oil. This product was stored and stored in a freezer.

Optical spectrum: \( \lambda_{\text{max}} \) = 302 and 333 nm in pH 7.0 water with a molar extinction coefficient of \( \sim 8400 \text{ M}^{-1} \text{ cm}^{-1} \) at the latter wavelength. ESI-MS (neg. mode) (75% ACN, 25% H\(_2\)O): Anion predicted (+H\(^+\)), 237.96 m/z; found, 239.94 m/z, 259.89 m/z (+Na\(^+\)), 339.04 m/z (+TEAH\(^+\)). Also observed, 162.17 m/z (N-acetylcysteine, impurity/decomposition).

Potassium Benzyl Trithiocarbonate (K[BnTTC]). The preparation was adapted from a known procedure. A round-bottom flask was charged with a magnetic stirring bar, diethyl ether (30 mL), and carbon disulfide (10 mL). The solution was cooled to 0–5 °C with an ice bath and then purged with nitrogen. Finely ground KOH (2.00 g, 35.6 mmol) was added, and then benzyl mercaptan (5 mL, 5.29 g, 42.6 mmol) was added to this cloudy mixture over a period of 2 min. The resulting solution rapidly became yellow and cloudy to give a chalky precipitate. This mixture was stirred for an additional 3 h. The solid product was collected by filtration and washed with diethyl ether and cold ethanol (4 x 10 mL). The solid was then added to a flask containing 150 mL of Et\(_2\)O and collected by filtration again to remove excess dimethylformamide. Recrystallization from a 50/50 mixture of ethanol and acetonitrile gave a deep yellow, mildly odorous powder that was dried in vacuo and stored in a freezer. The yield was 4.58 g (54%). K[BnTTC] decomposes when dissolved in water or water/alcohol mixtures to give highly odorous product(s). CHN anal. (calculated values for C\(_6\)H\(_7\)KS\(_3\) in parentheses): C, 39.8 (40.3); H, 2.84 (3.07). Optical spectrum: \( \lambda_{\text{max}} \) = 320 and 332 nm in pH 7.4 water (extinction coefficient of \( \sim 9300 \text{ M}^{-1} \text{ cm}^{-1} \) at the latter wavelength. (Note: Benzyl mercaptan has a particularly unpleasant odor and is toxic. Proper environmental controls must be observed when handling this liquid.)

**Kinetics Methods.** Phosphate buffers were prepared using mono- and dibasic sodium phosphate and sodium chloride (to maintain ionic strength). Nanopure water (\( \geq 18 \text{ megohm} \)) was obtained from a Barnstead Nanopure II system and used in solution preparations.

The rates of the TTC decompositions were determined by monitoring temporal spectral changes on a Shimadzu UV-2401 spectrophotometer with UVProbe kinetics software. The reaction cell was a septum-capped, sealable quartz cuvette with a 1.0 cm path length and an approximate volume of 4.80 mL. The cells were thermostated (\( \pm 0.2^\circ C \)) at the desired reaction temperature, and the solutions were stirred with a Starna “Spinette” stirrer. Septum-sealed cells containing the buffer solution of interest temperature equilibrated (7–10 min) under the desired conditions (typically 37 °C). A small amount of the solid TTC salt (5–10 mg) was then added to a vial containing 3–5 mL of buffer to prepare a stock solution. A 100 \( \mu L \) aliquot of this stock solution was then syringed into the thermostated cuvette. The volumes were chosen to minimize the headspace in the cuvette. Data acquisition was initiated after an estimated dead time of 60–90 s.

**Kinetics of the reactions of C\(_2\)S with various thiols to examine the rates of TTC formation were generally performed using an Applied Photophysics SX.19MV stopped-flow UV-visible spectrophotometer with photodiode array (temporal spectra) or a photomultiplier tube (single wavelength) detector. Aqueous buffer solutions of the thiol (RSH) and C\(_2\)S were loaded into Hamilton salt syringes (#1725 AD-SL), which were attached to the mixing block of the spectrophotometer and sealed from the outside atmosphere by a three-way valve. These syringes were maintained at the desired temperature (generally 37 °C unless otherwise noted) by a circulating water bath. The connector tubing and observation cell were purged with the sample solution by filling and emptying the drive syringes 3X prior to data acquisition. Stopped-flow mixing of the two solutions initiated the reaction. The entire unit was controlled with “Applied Photophysics Pro-Data SX” software. Only simultaneous symmetric mixing was used (1:1 dilution).

**Computational Studies.** All density functional theory (DFT) computations were performed using Gaussian’09 software packages. Optimizations were performed using Kohn-Sham DFT with the hybrid M06-2X exchange-correlation functional and the 6-311+G(\(d,p\)) basis set. No symmetry restriction was imposed, and implicit solvent effects were included using PCM (solvent = water) methods, as implemented in Gaussian 09. Vibrational frequency calculations were performed at the same level of theory to verify that no imaginary frequencies were present and to ensure the true local minima energies. Single-point energy and time-dependent DFT calculations were performed using the M062X/6-311+G(3df,3pd) level of theory.

**Analysis of C\(_2\)S Release.** This procedure was similar to that described by Schwach and Nyanzi and to that used in this laboratory to detect C\(_2\)S released by the photolysis of CdSe quantum dots surface decorated with 1,1-dithiooxalate and for the C\(_2\)S released in the thermal reactions of dithiocarbamate salts.

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**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Omega website at DOI: 10.1021/acsomega.7b01206.

Additional documentation (2 tables and 11 figures) of the studies (PDF)
**REFERENCES**

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The authors declare no competing financial interest. Carbon disulfide is toxic at high exposures, and long periods of modest exposure have been associated with a number of health conditions. CS₂ and CS₂-generating compounds should be handled and stored with care.

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**Notes**

The authors declare no competing financial interest. Carbon disulfide is toxic at high exposures, and long periods of modest exposure have been associated with a number of health conditions. CS₂ and CS₂-generating compounds should be handled and stored with care.

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(12) The SH group πK’s of N-acetylcyctine (NacSH) and 3-mercaptopropionic acid (3-MPA) are 9.5 and 10.3, respectively (refs 13 and 14). Benzyl mercaptan is not water soluble, but a value of 9.46 has been reported (ref 15) although since mercaptans are typically ~5.5 pKₐ units more acidic than the corresponding alcohols and benzyl alcohol has a pKₐ of 15.4 (ref 16), we would have estimated a slightly higher pKₐ of ~9.9.


