The Relative Rates of Thiol–Thioester Exchange and Hydrolysis for Alkyl and Aryl Thioalkanoates in Water

Paul J. Bracher · Phillip W. Snyder · Brooks R. Bohall · George M. Whitesides

Abstract This article reports rate constants for thiol–thioester exchange (k_{ex}), and for acid-mediated (k_a), base-mediated (k_b), and pH-independent (k_w) hydrolysis of S-methyl thioacetate and S-phenyl 5-dimethylamino-5-oxo-thiopentanoate—model alkyl and aryl thioalkanoates, respectively—in water. Reactions such as thiol–thioester exchange or aminolysis could have generated molecular complexity on early Earth, but for thioesters to have played important roles in the origin of life, constructive reactions would have needed to compete effectively with hydrolysis under prebiotic conditions. Knowledge of the kinetics of competition between exchange and hydrolysis is also useful in the optimization of systems where exchange is used in applications such as self-assembly or reversible binding. For the alkyl thioester S-methyl thioacetate, which has been synthesized in simulated prebiotic hydrothermal vents, $k_a = 1.5 \times 10^{-5}$ M^{-1}s^{-1}, $k_b = 1.6 \times 10^{-1}$ M^{-1}s^{-1}, and $k_w = 3.6 \times 10^{-8}$ s^{-1}. At pH 7 and 23°C, the half-life for hydrolysis is 155 days. The second-order rate constant for thiol–thioester exchange between S-methyl thioacetate and 2-sulfonatoethanethiolate is $k_{ex} = 1.7$ M^{-1} s^{-1}. At pH 7 and 23°C, with [R″S(H)] = 1 mM, the half-life of the exchange reaction is 38 h. These results confirm that conditions (pH, temperature, pK_a of the thiol) exist where prebiotically relevant thioesters can survive hydrolysis in water for long periods of time and rates of thiol–thioester exchange exceed those of hydrolysis by several orders of magnitude.

Keywords Thiol–thioester exchange · Thioesters · Origin of life · Prebiotic chemistry · Hydrolysis · Dynamic covalent chemistry

P. J. Bracher · P. W. Snyder · B. R. Bohall · G. M. Whitesides (✉)
Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA
e-mail: gwhitesides@gmwgroup.harvard.edu

Introduction

This paper describes the relative rates of thiol–thioester exchange and hydrolysis, as a function of the reaction conditions, for two unactivated thioesters in water. Under appropriate conditions (temperature, pH, pK_a of the thiol), the rate of thiol–thioester exchange can exceed the rate of hydrolysis of the thioester by several orders of magnitude. Our interest in aqueous reactions of thioesters centers on two subjects: (1) the plausibility of thioesters as thermodynamically activated compounds in prebiotic systems, and (2) the use of thiol–thioester exchange for applications in high-affinity reversible binding or self-assembly in water.

The ubiquity of thioesters in biology implicates them as prospective compounds of importance to the origin of life. Similar to the case of phosphoanhydrides (e.g., ATP), the hydrolysis of thioesters is relatively slow despite the large thermodynamic favorability of the reaction. Wächtershäuser, de Duve, and others have postulated that thioesters may have played a critical role in early metabolism, primarily as chemical reservoirs of energy (de Duve 1991; Huber and Wächtershäuser 1997, 1998; Wächtershäuser 1992). For these theories to have merit, conditions would have needed to exist where constructive reactions of thioesters could compete with hydrolysis to form biologically relevant products, even if only in low yield. Thiol–thioester exchange—the reaction between a thioester and a thiolate anion to produce a new thioester and thiolate as products—is one possible constructive reaction of thioesters on early Earth.

While common in biology, thiol–thioester exchange is less well understood—and much less used—in organic synthesis (Larsson, et al. 2004; Leclaire, et al. 2005; Maruyama and Hiraoka 1986) or reversible molecular assembly (Hadley and Gellman 2006; Woll and Gellman 2004). There are few reactions that generate covalent bonds reversibly in water (e.g., imine condensation, olefin metathesis, and thiol–disulfide interchange) (Corbett, et al. 2006; Rowan, et al. 2002). As a reversible reaction that proceeds in water in the range of pH relevant to biological processes, thiol–thioester exchange has the potential to be useful in processes based on self-assembly (Greig and Philp 2001; Rowan, et al. 2002).

For both prebiotic chemistry and molecular assembly, hydrolysis is a deleterious side reaction that prevents further reaction of the thioesters. Consequently, understanding the kinetics of hydrolysis as a function of the conditions is key to understanding the role of thioesters in these systems. Prior investigations into the kinetics of thioester exchange and hydrolysis generally examined electronically activated compounds (e.g., thioesters derived from trifluoroacetate) that are considerably more reactive than most of the thioesters found in biology and applied chemistry (Barnett and Jencks 1969; Fedor and Bruice 1965). We wished to examine thioesters of greater relevance to prebiotic chemistry and self-assembly. Previous studies have also required the use of organic co-solvents, because most simple organic thiols and thioesters are poorly soluble in aqueous buffers. Here, we examined compounds that have good solubility in water.

Background

In the thiol–thioester exchange reaction, a thiolate anion reacts with a thioester to form new thiolate and thioester products. In a competing process, thioesters spontaneously hydrolyze in water to form carboxylic acids (Fig. 1a). The relative rates of exchange and hydrolysis vary as a function of factors such as the pH of the medium and the acidities of the thiols. The kinetics of these reactions are important in the determination of optimal conditions for thiol–thioester exchange in water, where hydrolysis is a detrimental side reaction.

Fig. 1 **a** Schematic depictions of thiol–thioester exchange (**1A→1B**) and the hydrolysis of a thioester (**1A→1H**). For the generic thioester **1A**, thiol–thioester exchange to form thioester **1B** (reversible) and hydrolysis to form carboxylic acid **1H** (irreversible) are competing reactions in water. **b** The water-soluble thioesters and thiol investigated in this study
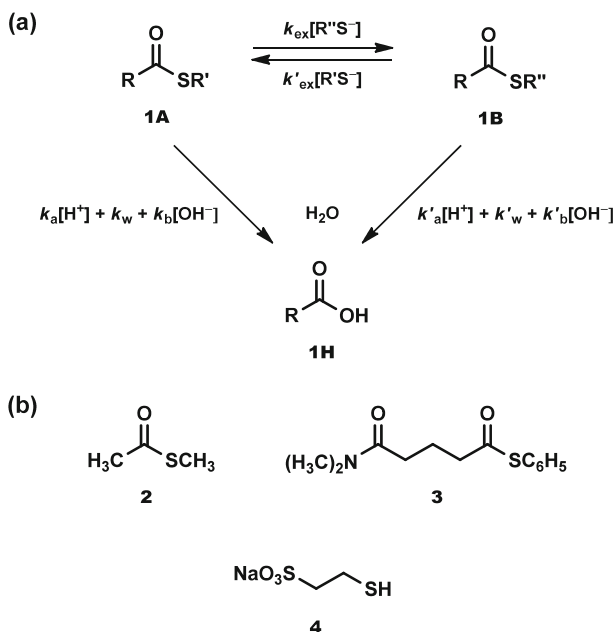
Castro has reviewed the kinetics and mechanisms of reactions of thioesters with nucleophiles in a variety of aqueous solvent systems (Castro 1999, 2007). The most thorough investigation into the kinetics of thiol–thioester exchange in water studied reactions between thioesters with pK_a(R′SH) of 2.75–7.91 and thiols with pK_a(R″SH) of 2.75–9.60 (Hupe and Jencks 1977). Hupe and Jencks found that the rate-determining step in the mechanism for thiol–thioester exchange is determined by the relative pK_a values of the incoming and departing thiols (R″SH and R′SH, respectively, in Fig. 1a). When the pK_a of the conjugate acid of the attacking thiolate is higher than that of the leaving thiolate, the rate-determining step is the formation of the tetrahedral intermediate. When the pK_a of the conjugate acid of the attacking thiolate is lower than that of the leaving thiolate, the rate-determining step is the break-down of the tetrahedral intermediate to form products. Figure 2 depicts possible mechanistic pathways for the hydrolysis of thioesters in water. Thioesters undergo both acid and base-mediated hydrolysis, though there are exceptions to this general rule (e.g., ethyl trifluorothiolacetate—for which acid *inhibits* hydrolysis) (Castro 1999, 2007; Jencks 1969). Equation 1 describes the pH–rate profile expected for a general thioester, where the observed rate of hydrolysis is the sum of three terms, which correspond to acid-mediated, pH-independent, and base-mediated processes:

$$\text{Rate}_{\text{hyd}} = (k_a[\text{H}^+] + k_w + k_b[\text{OH}^-])[\text{Thioester}] \tag{1}$$

While the hydrolysis of alkyl thioesters is thermodynamically favorable (e.g., for the hydrolysis of *S*-propyl thioacetate at 39°C and pH 7, $\Delta G \approx -7.7$ kcal·mol^{-1}) (Jencks, et al. 1960), reactions between thioesters and nucleophilic oxygen atoms are slow relative to reactions with nucleophilic sulfur atoms (Hupe and Jencks 1977).

The pH-independent rate of hydrolysis of unactivated thioesters is slow. The sluggishness of this hydrolysis may explain why values of k_w have been reported
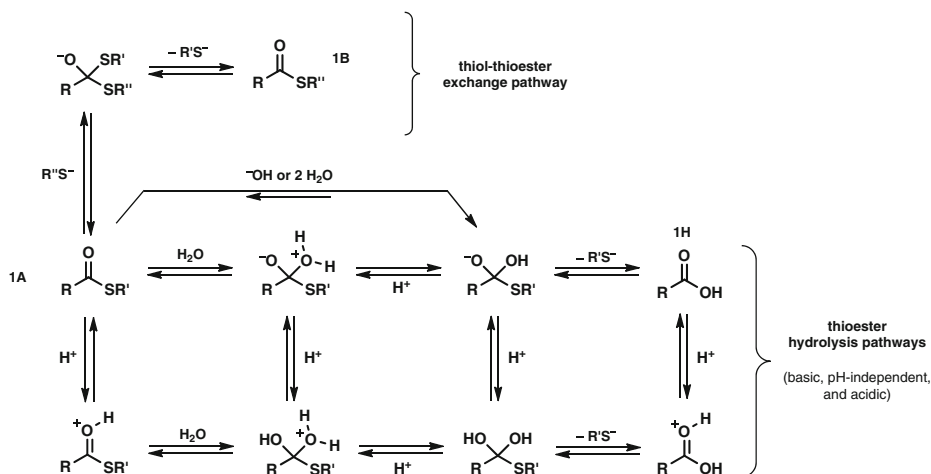
Fig. 2 Possible mechanistic pathways for thiol–thioester exchange (**1A**→**1B**, *top*) and the hydrolysis of a thioester (**1A**→**1H**, *bottom*) in water

only for electronically activated thioesters, such as derivatives of trifluoroacetic acid (Barnett and Jencks 1969; Fedor and Bruice 1965). *S*-phenyl thioformate—whose aryl thiol and formyl group render the thioester more reactive than the thioesters used in our study—is the least activated thioester for which the kinetics of pH-independent hydrolysis have been reported in the literature ($k_w = 3.3 \times 10^{-3}$ min.$^{-1}$, $t_{1/2} = 210$ min.) (Barnett and Jencks 1969).

Experimental Design

We wished to study the competition between thiol–thioester exchange and hydrolysis to: (1) evaluate to the potential importance of these reactions to prebiotic chemistry, and (2) calculate optimal conditions (values of pH, values of pK_a, and concentrations of the reactants) for using thiol–thioester exchange as a reversible, covalent basis for high-affinity binding in water.

In this study, we: (1) measured pseudo-first-order rate constants for hydrolysis of each thioester in the range pH 3–13 and [HCl] = 0–3 M to determine k_a, k_b, and k_w; (2) measured, independently, pseudo-first-order rate constants for thiol–thioester exchange over a convenient range of pH, to determine k_{ex}; (3) used these rate constants to calculate the ratio of the rates of exchange and hydrolysis at any value of pH as a function of the concentrations and pK_a values of the thiols.

NMR spectroscopy was a convenient method for monitoring the progress of the reactions. Concentrations of reactants were determined by comparing the areas of signals of the compounds to those of an internal standard in ^{1}H NMR spectra of the reaction mixtures. In a typical kinetics experiment, aliquots of the reaction mixtures were removed at timed intervals and dispensed into equal volumes of deuterated water. For reactions at high or low pH, the D$_2$O contained either acid (0.5–2% DCl) or base (saturated K$_2$HPO$_4$) to adjust the pH and slow the exchange and hydrolysis reactions until the sample could be analyzed by NMR spectroscopy. The final pH of each deuterated sample was selected such that neither

thiol–thioester exchange nor hydrolysis were detectable after 2 h (the maximum time between sampling the reaction mixture and obtaining the NMR spectrum of the aliquot).

We studied the hydrolysis of thioesters **2** and **3**, and their exchange with thiol **4** (see Fig. 1b for structures of these compounds). These thioesters and thiols were soluble in water and had at least one set of protons with a resonance signal completely resolved from the rest of the NMR spectrum. Losses in the intensity of these isolated signals (relative to an unreactive internal standard) were used to monitor the progress of the reaction over time. The compounds had water-soluble functional groups (dimethylamido and sulfonato) whose charges were independent of pH. We could use these compounds at high concentrations (5–200 mM) without influencing the pH of the solutions or exceeding the capacity of the buffers. Sodium benzenesulfonate and 2-methyl-2-propanol were selected as internal standards for the analysis by NMR spectroscopy because both are poor nucleophiles, have little effect on the pH of their solutions, and give rise to hydrogen signals that do not overlap with the hydrogen signals of the thiols and thioesters used in this study. The buffer concentrations were set to 200 mM to ensure sufficient capacity to hold the pH constant throughout the course of the hydrolysis.

Assumptions and Simplifications

The purpose of this study was to obtain a "first approximation" of the kinetics of hydrolysis and exchange with model compounds representative of unactivated thioesters. In the design of the experiments described here, we introduced simplifications to speed the investigation at the expense of experimental rigor. While the concentration of the buffer salts was fixed at 200 mM, the ionic strength of the solutions was not fixed at a constant value. This simplification could have created mismatches in the activities of the reactants between samples of differing pH, although we observed no significant deviations from the logarithmic scaling expected in the ranges dominated by acid- and base-mediated hydrolysis.

The alkaline hydrolysis of thioesters is governed by the kinetics of general base catalysis; the concentration of buffer—not just [OH$^-$]—affects the rate of the reaction (Jencks 1969). In order to account for changes in rate based on the different buffer salts used to prepare the samples, the reactions could have been performed at varying concentrations of buffer salt. Had we varied buffer concentration, the observed rate constants would have then been plotted against the concentration of buffer, and the expected k_{obs} values at zero buffer concentration would have been extrapolated for each pH. These are the values that would have been used to determine the rate constant k_b. We forwent these experiments to simplify the study by reducing the number of samples required to process.

In this study, we assumed that the attack of thiol (protonated thiolate) on the thioester did not contribute significantly to the rate of exchange. At values of pH near the pK_a of the thiol and far above the pK_a of the protonated carbonyl group, this assumption is probably valid. There is empirical support for the assumption: rates for the nucleophilic attack of mercaptans on ethylene oxide (another carbon electrophile) are proportional to the concentration of thiolate present (Danehy and Noel 1960). These authors noted k_{obs} scaled with [RS$^-$]/[RSH], which was essentially equal to [RS$^-$]/[RSH]$_{tot}$ over the ranges of pH studied (approximately 2–7 units below the pK_a values of the thiols). The assumption that thiolate is the only nucleophilic species involved in thiol–thioester exchange is expected to lead to increasing error as the pH decreases. Values of k_{ex} for R″SH (in addition to R″S$^-$) as the nucleophile should be measured if the model described here is to be suitable for the optimization of reaction conditions at low pH.

Hupe and Jencks found Brønsted relations between the rate of exchange and $pK_{a,\,nuc.}$ of the incoming thiol and $pK_{a,\,l.g.}$ of the outgoing thiol. The rate-determining step for the reaction depended on which thiol was more acidic. Instead of testing a range of thiols, we focused on reactions of 2-mercaptoethanesulfonate, a common water-soluble alkyl thiol. A systematic investigation of the dependence of k_{ex} on the acidity of the thiols would extend the model presented here by enabling the prediction of optimal pK_a values for thiols used in applications based on thiol–thioester exchange.

Results and Discussion

The rate of hydrolysis of a thioester varies as a function of pH. The values of the rate constants for acid-mediated hydrolysis (k_a), base-mediated hydrolysis (k_b), and pH-independent hydrolysis (k_w) can all be obtained experimentally. While the overall rate of hydrolysis of each thioester will vary with changes in pH, the values of these rate constants will not.

Figure 3 shows pH–rate profiles for the hydrolysis of thioesters **2** and **3**. The shapes of the profiles are consistent with the rate equation given in Eq. 1; both thioesters undergo predominant acid-mediated hydrolysis at low pH and predominant base-mediated hydrolysis at high pH. The intervening regions correspond to the predominance of pH-independent hydrolysis and are relatively low and flat. In theory, these regions should be perfectly flat. The deviations in these baselines from those expected from the elementary kinetic scheme, we assume, can be attributed to the experimental simplifications described previously (see Experimental Design section). In particular, (1) differences in the ionic strengths of the samples may have affected the activities of the reactants, and (2) the different buffer salts may have contributed unequally to the observed rates of hydrolysis—the rate constants were not extrapolated at zero buffer concentration by varying the concentration of buffer, and there may be small (but significant and different) contributions from the different buffer ions.

Determination of Rate Constants for Base-Mediated, Acid-Mediated, and pH-Independent Hydrolysis

If we assume that the pH of the buffered medium remains constant throughout the hydrolysis of each sample, then the terms $k_a[\mathrm{H^+}]$ and $k_b[\mathrm{OH^-}]$ will be constant and the hydrolysis will have pseudo-first-order kinetics. The assumption that the pH remains constant in these samples is reasonable; for the buffered samples (pH≥3), the concentration of thioester (**1A**) in these experiments is small relative to the concentration of the buffer salt (~1:20), and for the unbuffered samples (pH<1), the products of hydrolysis have $pK_a > 4$ and do not significantly influence the pH of the samples. The pH of the samples was measured at the beginning and end of each kinetics experiment to ensure there were no significant changes over the course of the runs. The plots of $-\ln([\mathbf{1A}]_t/[\mathbf{1A}]_0)$ versus time were all linear with Pearson correlation coefficients of $r^2 > 0.98$—further indication that the assumption of pseudo-first-order kinetics was valid.

Equation 2 is the integrated form of the rate law for the base-mediated, pseudo-first-order hydrolysis reaction.

$$\ln\left(\frac{[\mathbf{1A}]_t}{[\mathbf{1A}]_0}\right) = -k_b[\mathrm{OH^-}]\cdot t = -k_b\left(10^{\mathrm{pH}-14}\right)\cdot t \tag{2}$$
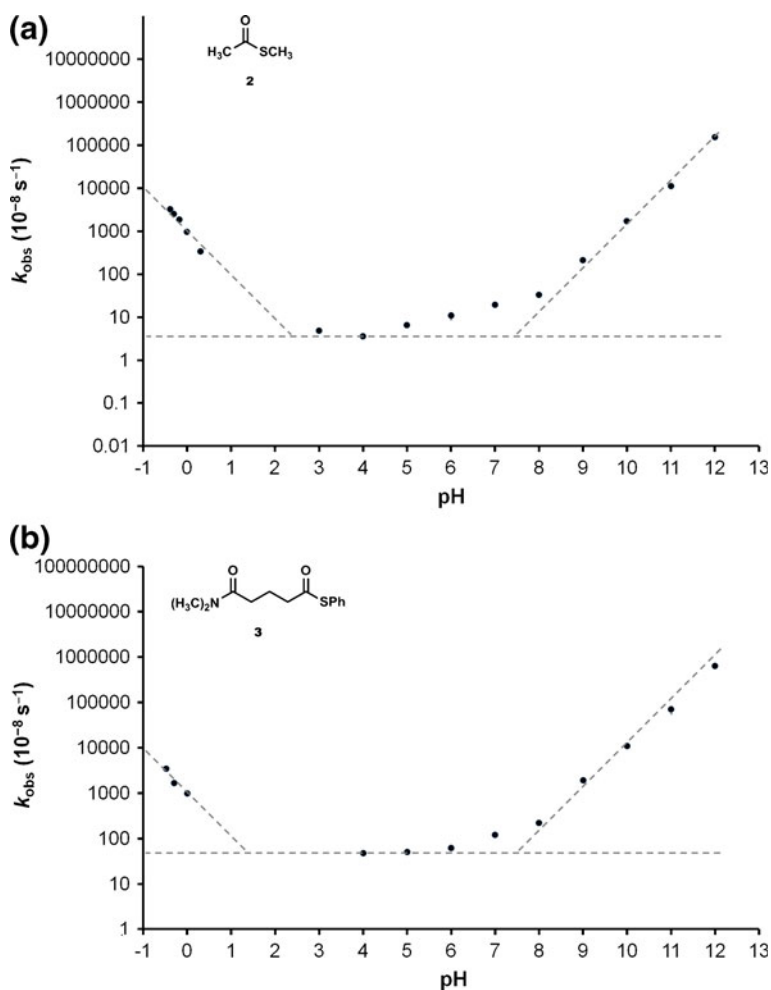
Fig. 3 Profiles of the observed rates of hydrolysis vs. pH at 23°C for (**a**) thioester **2** and (**b**) thioester **3**. The slanting *dashed lines* on the log-log plots have slopes of 1 and are meant to assist the reader in visualizing the regions where acid- and base-mediated hydrolysis predominate. The *horizontal dashed lines* mark $k_{w,obs}$, the minimum observed rate of hydrolysis for each thioester. The samples below pH 1 were unbuffered solutions of HCl. The samples above pH 1 were buffered with alkali salts of various anions (pH 3, 4: formate; pH 5, 6: pyrophosphate; pH 7, 8, 12, 13: phosphate; pH 9, 10, 11: carbonate) at concentrations of 200 mM. Each point represents the mean average of three trials and the error bars—some of which are small and difficult to resolve visually from the point markers—correspond to 95% confidence intervals. The rate constants were not extrapolated to zero buffer concentration and the ionic strength was not held constant across samples

When the hydrolysis of a thioester is monitored at constant pH in a regime where $k_b[OH^-] >> k_a[H^+] + k_w$, the value of k_b can be calculated by constructing a plot of $-\ln([1A]_t/[1A]_0)$ versus time (Fig. S3 in the Electronic Supplementary Material). The plot is linear with a slope of $k_b(10^{pH-14})$. The hydrolysis of each thioester was monitored at various values of pH, and the observed rates of hydrolysis were determined by constructing plots similar to Fig. S3. Base-mediated hydrolysis appears to be the predominant mode of

hydrolysis for *S*-methyl thioacetate (**2**) from pH 9 to 12. When the values for the observed rate of hydrolysis (k_{obs}, s^{-1}) over this range are plotted against the concentration of [OH$^-$] (M), the slope of the resulting line is equal to the second-order rate constant for base-mediated hydrolysis (k_b, M^{-1} s^{-1}). The data for the base-mediated hydrolysis of **2** are plotted in Fig. S4 in the Electronic Supplementary Material. For thioester **2**, $k_b = 1.6 \times 10^{-1}$ M^{-1}s^{-1}. For thioester **3**, $k_b = 6.4 \times 10^{-1}$ M^{-1}s^{-1}.

We determined the rate constants for acid-mediated hydrolysis (k_a) similarly in 0–3 M HCl. Buffered media were not required because the products of hydrolysis of the thioester (a thiol and carboxylic acid) have pK_a values much higher than the pH of the samples. Figure S5 (in the Electronic Supplementary Material) shows the data for the determination of the k_a of thioester **2**. For thioester **2**, $k_a = 1.5 \times 10^{-5}$ M^{-1}s^{-1}. For thioester **3**, $k_a = 1.2 \times 10^{-5}$ M^{-1}s^{-1}.

In straightforward cases where acid and base both accelerate—rather than inhibit—hydrolysis, the pH-independent pathway for hydrolysis represents a baseline to the overall observed rate of hydrolysis. The lowest observed rate constants for hydrolysis in the buffered solutions studied—$k_{obs} = 3.6 \times 10^{-8}$ s^{-1} for thioester **2** at pH 4 and $k_{obs} = 4.7 \times 10^{-7}$ s^{-1} for thioester **3** at pH 4—were taken as the values for k_w at 23°C. As expected from Eq. 1, these minimum observed rates both occurred in the intervening central regions of the pH–rate profiles (see Fig. 3).

Determination of the Second-Order Rate Constants for Thiol–Thioester Exchange

For thiol–thioester exchange in aqueous media, the rate of consumption of the starting thioester will correspond to the sum of the rates of exchange and hydrolysis, which occur simultaneously. If we subtract the rate of hydrolysis obtained in the previously-described experiments, we will be left with the rate of exchange. Equation 3 is the integrated form of the rate law for the consumption of a generic thioester, **1A**, by thiol–thioester exchange.

$$\ln\left(\frac{[\mathbf{1A}]_t}{[\mathbf{1A}]_0}\right) = -k_{ex}[RS^-] \cdot t = -k_{ex}\theta[RSH]_{tot} \cdot t, \tag{3}$$

where $\theta = \frac{1}{1+10^{pK_a - pH}}$

The expression for θ, the fraction of thiol present that is deprotonated at a given pH, is derived in the Electronic Supplementary Material. The rates of exchange exceeded hydrolysis by several orders of magnitude such that the latter had a negligible contribution to the consumption of thioester in our experiments used to measure k_{ex}. At constant pH, when a much higher concentration of thiol is present than of thioester, $\theta[RSH]_{tot}$ will be approximately constant over the course of the reaction and the kinetics will appear to be first-order. In a typical kinetics experiment for thiol–thioester exchange, aliquots were withdrawn at regular intervals from a mixture of thioester (5 mM), thiol (200 mM), 2-methyl-2-propanol (1.2 mM, internal standard), and pH buffer (200 mM). The aliquots were immediately diluted with equal volumes of 0–1.5% DCl in D$_2$O to quench the reaction and provide a deuterated solvent for subsequent analysis by NMR spectroscopy.

When the reaction was monitored in a buffered medium of constant pH, the value of k_{ex} could be calculated by constructing a plot of $-\ln([\mathbf{1A}]_t/[\mathbf{1A}]_0)$ versus time, as shown in Fig. S6 in the Electronic Supplementary Material (with data from the exchange of *S*-methyl thioacetate, **2**, with 2-mercaptoethanesulfonate, **4**, at pH 6). The plot is linear with a slope of $k_{ex}\theta[RSH]_{tot}$. The exchange reaction of each thioester was monitored at various levels of pH. The observed rates of exchange were determined by constructing plots similar to

Fig. S6. For exchange with the conjugate base of thiol **4**, we measured $k_{ex} = 1.7$ M^{-1} s^{-1} for thioester **2** and $k_{ex} = 1.2 \times 10^2$ M^{-1} s^{-1} for thioester **3**.

Profiles of the Relative Rates of Exchange and Hydrolysis

Table 1 summarizes the rate constants we determined experimentally. Figure 4 shows computed pH–rate profiles for the hydrolysis of thioesters **2** and **3** and for their exchange with **4** based on the measured values of k_a, k_w, k_b, and k_{ex}. These plots illustrate several key features of the systems. The blue lines correspond to the rates of pH-independent hydrolysis, and hence, they are flat. The rates of alkaline (green) and acidic (purple) hydrolysis scale with the concentrations of OH$^-$ and H$^+$, respectively. Finally, the rates of exchange (red) scale with the concentrations of thiolate, which is why the rates plateau shortly after pH = pK_a(R″SH). While the shapes of the plots are similar for both thioesters, the rates of exchange, base-mediated hydrolysis, and pH-independent hydrolysis are all greater for the aryl thioester **3** than alkyl thioester **2**. The rate of acid-catalyzed hydrolysis is slightly lower for **3** than **2**. These results could be explained by: (1) the inductive effect of the aryl group—the aromatic ring withdraws negative charge density from the carbonyl group, rendering it more susceptible to attack by electrophiles and less susceptible to protonation, and (2) aromatic thiolates are better leaving groups than alkyl thiolates.

Figure 5 plots the (computed) relative rates of exchange versus hydrolysis for thioesters **2** and **3** with thiol **4** based on our experimentally determined rate constants. The rate of exchange with **4** is maximized relative to hydrolysis at pH 8.4 and 8.7 for thioesters **2** and **3**, respectively. Note that while these values are similar—because the thiol remains the same in both systems—the relative rate of exchange to hydrolysis is over an order of magnitude greater at the optimal pH for **3** than **2**. This difference reflects the observation that the rate of exchange (with thiol **4**) for **3** exceeded that of **2** by roughly two orders of magnitude while the rates of alkaline and pH-independent hydrolysis were only one order of magnitude greater for **3** than **2**.

Another parameter open to control in these systems is the concentration of thiol. The relative rate of exchange vs. hydrolysis can be increased by raising the concentration of thiol present. The rate of exchange scales linearly with [RSH]$_{tot}$, while the rate of hydrolysis remains constant. In the calculations presented here, unless otherwise noted, the concentration of thiol has been arbitrarily set to 1 mM.

Table 1 Rate constants measured for the hydrolysis of thioesters **2** and **3**, and for their exchange with thiol **4**. The observed rate of hydrolysis, k_{obs}, resolves into terms corresponding to acid-mediated, pH-independent, and base-mediated processes in the expression $k_{obs} = k_a[H^+] + k_w + k_b[OH^-]$. The second-order rate constant for thiol–thioester exchange corresponds to the reaction of the thioester with 2-sulfonatoethanethiolate (the conjugate base of **4**). The rate expression is: rate$_{ex} = k_{ex}[RS^-]$[thioester] $= k_{ex}\theta[4]_{tot}$[thioester]

	Rate constants			
	k_a (M^{-1} s^{-1})	k_w (s^{-1})	k_b (M^{-1} s^{-1})	k_{ex} (M^{-1} s^{-1})
Thioester **2**	1.5×10^{-5}	3.6×10^{-8}	1.6×10^{-1}	1.7×10^0
Thioester **3**	1.2×10^{-5}	4.7×10^{-7}	6.4×10^{-1}	1.2×10^2

Fig. 4 Profiles of the calculated pseudo-first-order rate constants for acid-mediated hydrolysis ($k_{a,\,obs} = k_a[H^+]$, *purple*), base-mediated hydrolysis ($k_{b,\,obs} = k_b[OH^-]$, *green*), pH-independent hydrolysis ($k_{w,\,obs} = k_w$, *blue*), and thiol–thioester exchange ($k_{ex,\,obs} = k_{ex}[R''S^-]$, *red*) of thioester **2** (plot a), thioester **3** (plot b) with 2-mercaptoethanethiol ($pK_{a,\,nuc.} = 9.53$). The *black line* represents the total observed pseudo-first-order rate constant for hydrolysis—the sum of the acid-catalyzed, base-mediated, and pH-independent processes. The concentration of thiol, $[R''SH]$, was arbitrarily set to 1 mM
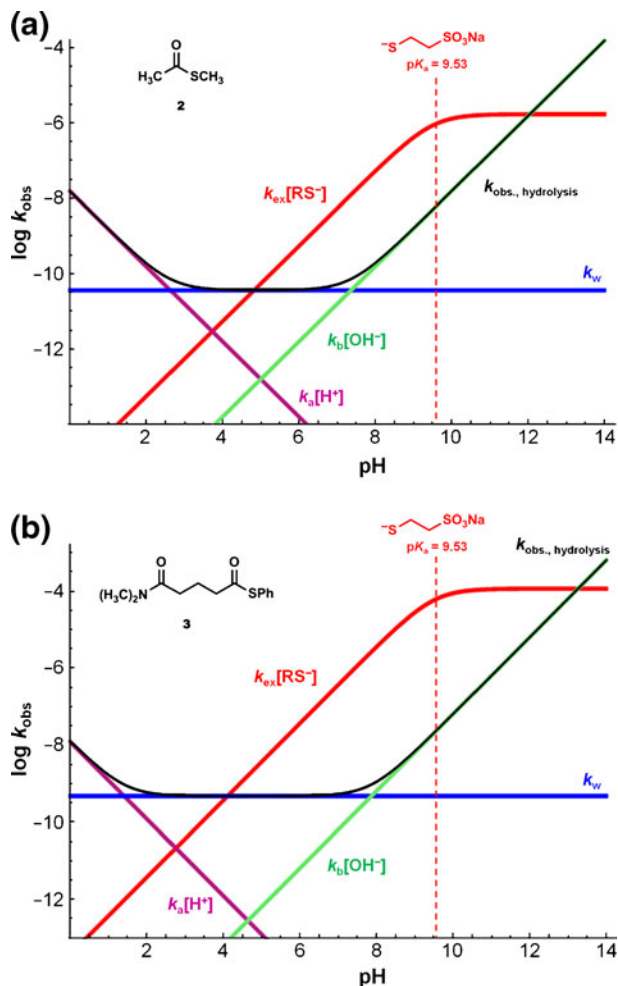

Implications for Thioesters in the Origin of Life

Thioesters are commonly encountered in biochemistry, e.g., as derivatives of coenzyme A, appendages on the pantetheine arms of acyl carrier protein in the biosynthesis of fatty acids, and intermediates during the hydrolysis of peptide bonds by cysteine proteases (Bruice and Benkovic 1966; Carreras, et al. 1997; Otto and Schirmeister 1997). The ubiquity of thioesters in biology suggests their early inclusion in biochemical processes and that they may have played an important role in the origin of life. For thioesters to have contributed to the development of life on Earth, constructive reactions that could have generated molecular complexity would have needed to compete effectively with destructive reactions—especially, hydrolysis.

Classes of constructive processes possibly important for the origin of life include those that (1) build large molecules from smaller reagents, (2) concentrate and sequester molecules of biological importance, and (3) enable the storage and transfer of energy. Thiol–thioester exchange is one example of a reaction that can play central roles in

Fig. 5 Plots of the calculated ratio of the rates of thiol–thioester exchange and hydrolysis as a function of pH for (a) S-methyl thioacetate (2) and (b) S-phenyl 5-dimethylamino-5-oxo-thiopentanoate (3) with alkyl thiol 4 ($pK_{a, \text{nuc.}}$ = 9.53). The concentration of thiol, [R″SH], was arbitrarily set to 1 mM
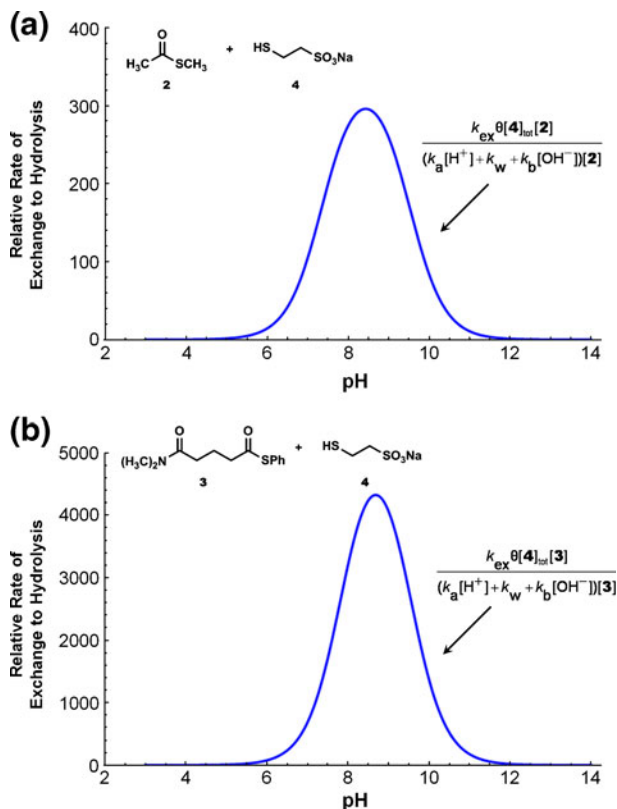
constructive processes from all three of these classifications. Monomers functionalized with both thiols and thioesters can polymerize into macromolecules (Ura, et al. 2009). Reactions coupled to the hydrolysis of thioesters (e.g., formation of phosphoanhydrides via acyl-phosphate intermediates) can be used to store energy (de Duve 1991; Wächtershäuser 1992). Solid phases functionalized with thiols could be used to concentrate thioester-functionalized molecules on surfaces and catalyze reactions (Wächtershäuser 1992).

Various experimental studies have reported the synthesis of thioesters under possible primordial conditions (Weber 1984, 1998). S-methyl thioacetate (2) is the simplest example of a thioester, and the kinetics of its hydrolysis are especially pertinent to origin-of-life chemistry. The production of 2—followed by its immediate hydrolysis—has been observed in simulated hydrothermal vents containing (Ni, Fe)S, CO, and CH_3SH (Huber and Wächtershäuser 1997, 1998).

For thioesters to have been important molecules to the development of life on early Earth, conditions would have needed to exist where constructive reactions could compete with the destructive reaction of hydrolysis. To our knowledge, no study has reported rates of hydrolysis for unactivated thioesters at mild pH, while at least one paper on origin-of-life chemistry has posited that "thioesters have short half-lives in neutral water" (Committee on the Limits of Organic Life in Planetary Systems, et al. 2007). This qualitative statement is misleading, if not outright wrong. Based on our kinetics studies, the half-life for hydrolysis of S-methyl thioacetate (2) in water of pH 7 is 155 days at 23°C. For the aryl thioester 3, the half-life is 15 days. In contrast, the half-life for thiol–thioester exchange with thiol 4 (1 mM) is 38 h for thioester 2, and 34 min for thioester 3.

The acidity of prebiotic bodies of water would have been an important consideration in determining the lifetime of thioesters on early Earth. While the current ocean has a pH of 7.8 (Von Damm, et al. 1997), prebiotic bodies of water are thought to have been more acidic, due to the high partial pressure of CO_2 in the ancient atmosphere. The sedimentary rock record is consistent with high ancient partial pressures of CO_2 and a more acidic Archean ocean (Walker 1983). Krupp, et al. estimated the Archean ocean to be pH 6.4–7 based on the assumed establishment of a bicarbonate-carbonic acid buffer (Krupp, et al. 1994). The same authors estimated a pH as low as 3.9 for Archean rain and surface water, based on the pH of water saturated in carbonic acid under an atmosphere containing $p(CO_2) = 1$ bar. At pH 6.4 and 23°C, the half-life of thioester **2** is 200 days. At pH 3.9, the half-life is 211 days. In acid, thiol–thioester exchange proceeds slowly for the attack of alkyl thiols because these conditions generate only a small proportion of thiolate (the active nucleophile). A mildly acidic prebiotic ocean would have favored thioester exchange with more acidic thiols (e.g., thiophenol, PhSH, $pK_a \approx 7$) than alkyl thiols (e.g., 2-sulfonatoethanethiol, thiol **4**, $pK_a = 9.5$). For more acidic thiols than **4**, the optimal values of pH for exchange (the maxima of the curves in Fig. 5) would be shifted lower.

The existence of an acidic prebiotic ocean would not necessarily have excluded base-catalyzed processes (like thiol–thioester exchange) on the young planet. Off-ridge, oceanic hydrothermal springs could have provided local aqueous environments on early Earth that were alkaline rather than acidic (Macleod, et al. 1994). The recently discovered Lost City vent field of the mid-Atlantic Ocean has a pH range of 9–11, which stands in stark contrast to the acidic output typical of "black smoker" vents (Kelley, et al. 2005). Thiol–thioester exchange for alkyl thiols would have been rapid within this range (Fig. 4).

We see no reason—based on the kinetics of hydrolysis—to reject the idea that thioesters may have been important for the development of life in an aqueous environment on early Earth. This study demonstrates that thioesters can survive for hundreds of days in water and that productive reactions of thioesters can compete effectively with hydrolysis. We note that this model does not account for the possible effects of reactive solutes (such as metals) that would have been present in the prebiotic ocean.

Implications for the Use of Thiol–Thioester Exchange as a Reversible, Covalent Interaction

Reactions that take place in water in high yield are particularly appealing for their potential use in biological applications. Thiol–thioester exchange can proceed in high yield in water, near neutral pH, and at room temperature. An example of the utility of the reaction is its role as the first step in the mechanism of native chemical ligation (NCL). In NCL, a peptide is synthesized from a smaller peptide fragment with a C-terminal thioester and a fragment that bears an N-terminal cysteine residue (Dawson and Kent 2000; Dawson, et al. 1994). Dawson and coworkers have shown that the addition of thiols can be used to modulate the reactivity of the peptide–α-thioester by thiol–thioester exchange (Dawson, et al. 1997).

The reversibility of thiol–thioester exchange and its tolerance for other functional groups suggest that the reaction could serve as a covalent alternative to non-covalent interactions useful in self-assembly or reversible binding in water. In order for a binding system based on thiol–thioester exchange to be useful in practical applications, the conditions selected for the reaction must favor a fast rate of exchange and minimal hydrolysis. For the generic thioester **1A**, the optimal pH for thiol–thioester exchange is the pH where the ratio of the rate of exchange to the rate of hydrolysis is highest. This pH is determined by k_w, k_a, k_b, and $pK_a(R''SH)$; it is independent of k_{ex}, total concentration of thiol, and concentration of thioester (King, et al. 1992). The concentration of thioester ([**1A**]) does not affect the

optimal pH because both the rates of exchange and of hydrolysis are first-order in [**1A**], so the concentration terms cancel. Our study verifies that thiol–thioester exchange could be useful in the design of binding systems, and the analysis here provides a framework for the design and optimization of these systems. The construction of pH–rate profiles (e.g., Figs. 4 and 5) from the rate laws and measured rate constants (k_{ex}, k_a, k_b, and k_w) allows the determination of how the relative rates of exchange and hydrolysis vary as a function of changes in the pH of the solution and pK_a of the thiols. This study is the first to report rate constants for pH-independent hydrolysis (k_w) of unactivated thioesters in water. Accurate values for these rate constants are critical in constructing pH–rate profiles for the optimization of thioester exchange versus hydrolysis.

Conclusions

The rates of thiol–thioester exchange and hydrolysis for a thioester are pH dependent, and measurement of the rate constants k_{ex}, k_a, k_b, and k_w enables the determination of conditions where the rate of exchange is maximized relative to hydrolysis. Similar calculations should prove useful in the design of systems that employ thiol–thioester exchange as a reversible binding event in aqueous media, where hydrolysis is a deleterious side reaction. The analysis presented here allows the determination of an optimum pH for the reaction between a particular thiol–thioester pair. The results demonstrate that conditions exist where the rate of thiol–thioester exchange exceeds the rate of hydrolysis by several orders of magnitude. With regard to the origin of life, we see no reason—on the basis of the kinetics of hydrolysis—for blanket skepticism or rejection of theories that invoke thioesters as molecules of prebiotic importance.

References

Barnett R, Jencks WP (1969) The rates of hydrolysis of two thiol esters in water. J Org Chem 34:2777–2779. doi:10.1021/jo01261a070

Bruice TC, Benkovic SJ (1966) Bioorganic mechanisms: volume I. W.A. Benjamin, Inc, New York

Carreras CW, Pieper R, Khosla C (1997) The chemistry and biology of fatty acid, polyketide, and nonribosomal peptide biosynthesis. In: Bioorganic chemistry deoxysugars, polyketides and related classes: synthesis, biosynthesis, enzymes. Springer-Verlag Berlin, Berlin 33, pp. 85–126

Castro EA (1999) Kinetics and mechanisms of reactions of thiol, thiono, and dithio analogues of carboxylic esters with nucleophiles. Chem Rev 99:3505–3524. doi:10.1021/cr990001d

Castro EA (2007) Kinetics and mechanisms of reactions of thiol, thiono, and dithio analogues of carboxylic esters with nucleophiles. An update. J Sulfur Chem 28:401–429. doi:10.1080/17415990701415718

Committee on the Limits of Organic Life in Planetary Systems, Committee on the Origins and Evolution of Life, The National Research Council (2007) The limits of organic life in planetary systems. The National Academies, Washington

Corbett PT, Leclaire J, Vial L, West KR, Wietor JL, Sanders JKM, Otto S (2006) Dynamic combinatorial chemistry. Chem Rev 106:3652–3711. doi:10.1021/cr020452p

Danehy JP, Noel CJ (1960) The relative nucleophilic character of several mercaptans toward ethylene oxide. J Am Chem Soc 82:2511–2515. doi:10.1021/ja01495a028

Dawson PE, Kent SBH (2000) Synthesis of native proteins by chemical ligation. Annu Rev Biochem 69:923–960. doi:10.1146/annurev.biochem.69.1.923

Dawson PE, Muir TW, Clark-Lewis I, Kent SBH (1994) Synthesis of proteins by native chemical ligation. Science 266:776–779

Dawson PE, Churchill MJ, Ghadiri MR, Kent SBH (1997) Modulation of reactivity in native chemical ligation through the use of thiol additives. J Am Chem Soc 119:4325–4329. doi:10.1021/ja962656r

de Duve C (1991) Blueprint for a cell. Neil Patterson Publishers, Burlington

Fedor LR, Bruice TC (1965) Nucleophilic displacement reactions at the thiol ester bond. IV. General base catalyzed hydrolysis of ethyl trifluoroacetate. Kinetic evidence for the formation of a tetrahedral intermediate. J Am Chem Soc 87:4138–4147. doi:10.1021/ja01096a024

Greig LM, Philp D (2001) Applying biological principles to the assembly and selection of synthetic superstructures. Chem Soc Rev 30:287–302. doi:10.1039/b104962n

Hadley EB, Gellman SH (2006) An antiparallel alpha-helical coiled-coil model system for rapid assessment of side-chain recognition at the hydrophobic interface. J Am Chem Soc 128:16444–16445. doi:10.1021/ja067178r

Huber C, Wächtershäuser G (1997) Activated acetic acid by carbon fixation on (Fe, Ni)S under primordial conditions. Science 276:245–247. doi:10.1126/science.276.5310.245

Huber C, Wächtershäuser G (1998) Peptides by activation of amino acids with CO on (Ni, Fe)S surfaces: implications for the origin of life. Science 281:670–672. doi:10.1126/science.281.5377.670

Hupe DJ, Jencks WP (1977) Nonlinear structure-reactivity correlations. Acyl transfer between sulfur and oxygen nucleophiles. J Am Chem Soc 99:451–464. doi:10.1021/ja00444a023

Jencks WP (1969) Catalysis in chemistry and enzymology. McGraw-Hill, Inc, New York

Jencks WP, Cordes S, Carriuolo J (1960) Free energy of thiol ester hydrolysis. J Biol Chem 235:3608–3614

Kelley DS, Karson JA, Fruh-Green GL, Yoerger DR, Shank TM, Butterfield DA, Hayes JM, Schrenk MO, Olson EJ, Proskurowski G, Jakuba M, Bradley A, Larson B, Ludwig K, Glickson D, Buckman K, Bradley AS, Brazelton WJ, Roe K, Elend MJ, Delacour A, Bernasconi SM, Lilley MD, Baross JA, Summons RT, Sylva SP (2005) A serpentinite-hosted ecosystem: the lost city hydrothermal field. Science 307:1428–1434. doi:10.1126/science.1102556

King JF, Rathore R, Lam JYL, Guo ZR, Klassen DF (1992) pH optimization of nucleophilic reactions in water. J Am Chem Soc 114:3028–3033. doi:10.1021/ja00034a040

Krupp R, Oberthür T, Hirdes W (1994) The early precambrian atmosphere and hydrosphere: thermodynamic constraints from mineral deposits. Econ Geol 89:1581–1598. doi:10.2113/gsecongeo.89.7.1581

Larsson R, Pei ZC, Ramström O (2004) Catalytic self-screening of cholinesterase substrates from a dynamic combinatorial thioester library. Angew Chem Int Ed 43:3716–3718. doi:10.1002/anie.200454165

Leclaire J, Vial L, Otto S, Sanders JKM (2005) Expanding diversity in dynamic combinatorial libraries: simultaneous exchange of disulfide and thioester linkages. Chem Commun: 1959–1961. doi:10.1039/b500638d

Macleod G, McKeown C, Hall AJ, Russell MJ (1994) Hydrothermal and oceanic pH conditions of possible relevance to the origin of life. Orig Life Evol Biosph 24:19–41

Maruyama H, Hiraoka T (1986) A stereocontrolled synthesis of thienamycin from 6-aminopenicillanic acid. J Org Chem 51:399–402. doi:10.1021/jo00353a025

Otto HH, Schirmeister T (1997) Cysteine proteases and their inhibitors. Chem Rev 97:133–171. doi:10.1021/cr950025u

Rowan SJ, Cantrill SJ, Cousins GRL, Sanders JKM, Stoddart JF (2002) Dynamic covalent chemistry. Angew Chem Int Ed 41:898–952. doi:10.1002/1521-3773(20020315)41:6<898::AID-ANIE898>3.0.CO;2-E

Ura Y, Al-Sayah M, Montenegro J, Beierle JM, Leman LJ, Ghadiri MR (2009) Dynamic polythioesters via ring-opening polymerization of 1,4-thiazine-2,5-diones. Org Biomol Chem 7:2878–2884. doi:10.1039/b903612a

Von Damm KL, Buttermore LG, Oosting SE, Bray AM, Fornari DJ, Lilley MD, Shanks WC III (1997) Direct observation of the evolution of a seafloor 'black smoker' from vapor to brine. Earth Planet Sci Lett 149:101–111

Wächtershäuser G (1992) Groundworks for an evolutionary biochemistry—the iron sulfur world. Prog Biophys Mol Biol 58:85–201

Walker JCG (1983) Possible limits on the composition of the Archean ocean. Nature 302:518–520. doi:10.1038/302518a0

Weber AL (1984) Prebiotic formation of energy-rich thioesters from glyceraldehyde and N-acetylcysteine. Orig Life Evol Biosph 15:17–27. doi:10.1007/BF01809390

Weber AL (1998) Prebiotic amino acid thioester synthesis: thiol-dependent amino acid synthesis from formose substrates (formaldehyde and glycolaldehyde) and ammonia. Orig Life Evol Biosph 28:259–270. doi:10.1023/A:1006524818404

Woll MG, Gellman SH (2004) Backbone thioester exchange: a new approach to evaluating higher order structural stability in polypeptides. J Am Chem Soc 126:11172–11174. doi:10.1021/ja046891i