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Nucleophilic Catalysis of Hydrazone Formation and Transimination: Implications for Dynamic Covalent Chemistry

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Dynamic covalent chemistry (DCC) has great potential in the fields of drug discovery and materials science.[1−4] However, its impact has been tempered by the slow equilibration kinetics of bonds that meet the stability requirements for these applications. A typical example, widely explored for DCC, is the hydrazone reaction.[4−7] The reaction between a hydrazide and a carbonyl is chemoselective, and the equilibrium favors the hydrazone in aqueous solution. However, its equilibration kinetics are slow. To increase the dynamics of the hydrazone reaction without perturbing its chemical equilibrium, a transimination catalyst is required.[8] Recently, we have shown that aniline effectively accelerates oxime ligation by forming in situ a highly reactive protonated aniline Schiff base.[9] In a similar fashion, aniline should catalyze both hydrazone formation and hydrolysis, which would facilitate rapid transimination of hydrazones under aqueous conditions. Here we show that the equilibration kinetics of hydrazone formation and transimination can be significantly accelerated by using aniline as a nucleophilic catalyst.

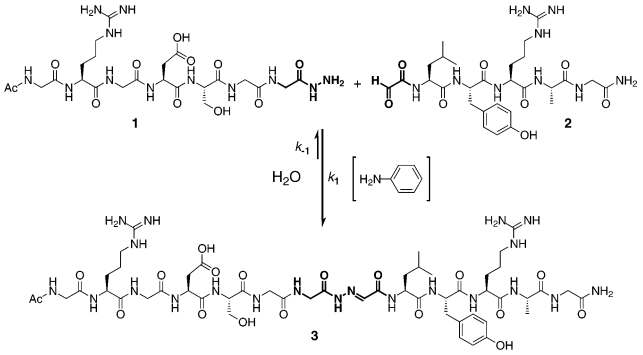
To investigate the effect of aniline on the rates of hydrazone formation (k_1) and hydrolysis (k_{-1}), two unprotected peptides, AcGRGDSGG−hydrazide **1** and glyoxylyl−LYRAG **2**, were reacted at pH 5.7 in the presence and absence of 10 mM aniline at ambient temperature using equimolar amounts of the peptides (Scheme 1). The reactions were followed by HPLC, and ligation product **3** was quantitated by integration (214 nm).

As shown in Figure 1, aniline significantly catalyzes the equilibration of the hydrazone reaction. Fitting the data for the ligation at 1 mM peptide concentration to the rate equation for a reversible second-order reaction (see Supporting Information) revealed a ∼70-fold rate enhancement in the second-order rate constant k_1 from 0.0031 ± 0.0001 to 0.21 ± 0.01 M^{-1} s^{-1}, while the pseudo-first-order rate constant k_{-1} increased accordingly from $(0.11 \pm 0.04) \times 10^{-6}$ to $(4.0 \pm 0.6) \times 10^{-6}$ s^{-1}. As expected for a nucleophilic catalyst, aniline did not significantly affect the equilibrium constant of the reaction. In addition, the rate enhancement was independent of the reactant concentration; for example, at 0.1 mM peptide concentration, k_1 is also increased ∼70-fold from 0.0029 ± 0.0002 to 0.21 ± 0.03 M^{-1} s^{-1} in the presence of 10 mM aniline.

Hydrazone reactions are typically fastest at approximately pH 4.5.[10] At this pH, the rates of the uncatalyzed reaction at 1 mM peptide concentration are ∼10-fold faster than those at pH 5.7, $k_1 = 0.030 \pm 0.002$ M^{-1} s^{-1} and $k_{-1} = (0.1 \pm 0.1) \times 10^{-5}$ s^{-1}. Under these more acidic conditions, the addition of 10 mM aniline ($pK_a = 4.6$)[11] still results in a ∼20-fold rate enhancement, $k_1 = 0.49 \pm 0.02$ M^{-1} s^{-1} and $k_{-1} = (2.6 \pm 0.4) \times 10^{-5}$ s^{-1}.

Aniline-mediated transimination is expected to proceed through an aniline Schiff base intermediate. However, under the reaction conditions applied, aniline Schiff base intermediates are not observed by either HPLC or direct electrospray ionization mass spectrometry analysis of the reaction mixture, presumably due to their small K_{eq}.[11] Direct evidence for the presence of the aniline Schiff base at equilibrium was obtained from an attempt to reduce the hydrazone product **3** (Scheme 2). A 10-fold excess of $NaBH_3CN$ was added to an equimolar ligation mixture of **1** and **2** (1 mM) in equilibrium with **3** (80% at equilibrium) in the presence of 10 mM aniline at pH 4.5. Instead of yielding the expected acyl hydrazide peptide **7**, a near quantitative yield of the aniline reductive alkylation product **6** was obtained. This result is explained by the pK_a difference between the aniline Schiff base **4** ($pK_a \sim 3$)[12] and the hydrazone **3** (pK_a between 0 and −3);[13] despite the low abundance of the aniline Schiff base in solution, it is the major protonated imine present at equilibrium. Since $NaBH_3CN$ reacts exclusively with protonated imines,[14] the rapid and irreversible

Scheme 1. Hydrazone Reaction of AcGRGDSGG−Hydrazide **1** and Glyoxylyl−LYRAG **2** in the Absence or Presence of Aniline



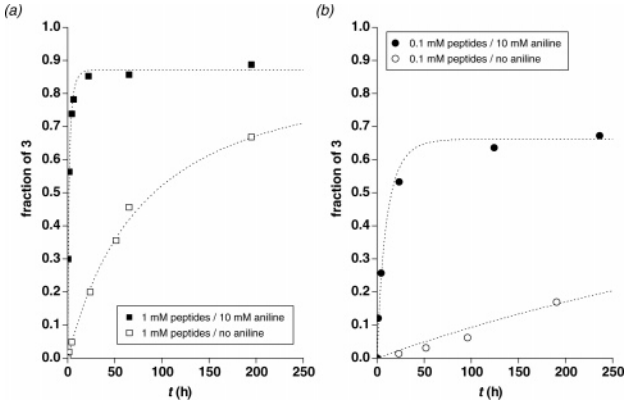


Figure 1. Formation of hydrazone **3** over time (a) at 1 mM and (b) 0.1 mM reactant concentration at room temperature in 0.1 M NH_4OAc buffer (pH 5.7) in the absence (□/○) and in the presence (■/●) of 10 mM aniline. The dotted lines represent the fit of the data to the rate equation (see Supporting Information).

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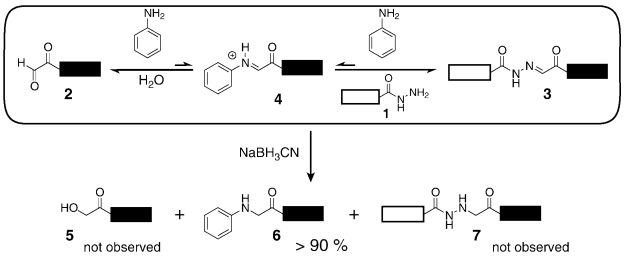
Scheme 2. Covalent Capture of the Aniline Schiff Base **4** from a Pre-equilibrated Mixture of Hydrazide **1**, Glyoxylyl **2**, Hydrazone **3**, and Aniline through Reduction with $NaBH_3CN$ ($[\mathbf{1}]_0 = [\mathbf{2}]_0 = 1$ mM; 10 mM $NaBH_3CN$, 0.1 M NH_4OAc, pH 4.5, RT, 25 h): A Small Level of **3** Remained Unreacted



reduction of the small population of **4** leads to an almost full conversion of the hydrazone into the aniline-modified peptide **6**.

The ongoing exchange between aniline and the hydrazone at equilibrium suggests that aniline should catalyze transimination, the exchange reaction of hydrazones with other amines in solution. At high concentration, the time to reach a new equilibrium will be predominantly determined by the rate of hydrolysis (k_{-1}). For example, at 1 mM reactants, the back reaction is 1 order of magnitude slower than hydrazone formation (k_1). Without a catalyst, only low levels of transimination have been achieved, and elevated temperatures would be required to accelerate the equilibration kinetics.

To illustrate the utility of nucleophilic catalysis for hydrazone transimination, an exchange experiment was performed between hydrazone **3** and a competing hydrazide **8** (Figure 2). First, an equimolar solution of hydrazide **2** and glyoxylyl **1** (1 mM each, 10 mM aniline) was equilibrated to yield a mixture of 0.8 mM hydrazone **3** and 0.2 mM each of hydrazide **1** and glyoxylyl **2** (Figure 2A). To this solution was added 1 equiv of hydrazide **8**, and the aniline concentration was increased to 20 mM. In just 10 h, a new equilibrium was established, composed of hydrazones **3** and **9** in a 1:1.2 molar ratio (Figure 2B), indicating similar hydrazone stability. Finally, 1.1 equiv of aminooxyacetyl−LYRAG **10** (100 mM aniline) was added to this dynamic mixture, shifting the equilibrium toward the oxime **11** (80%) (Figure 2C).[15] Although the hydrazides have a greater k_1, over time, the thermodynamically more stable oxime **11** is obtained due to its slower k_{-1}.

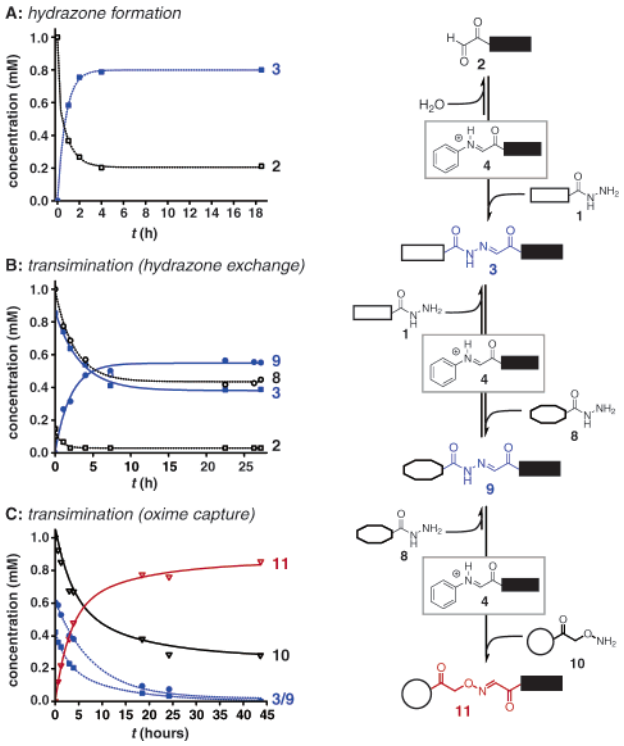


Figure 2. Aniline-catalyzed hydrazone formation (A: $[\mathbf{1}]_0 = [\mathbf{2}]_0 = 1$ mM, [aniline] = 10 mM) and transimination (B: $[\mathbf{8}]_0 = 1$ mM, [aniline] = 20 mM; C: $[\mathbf{10}]_0 = 1.1$ mM, [aniline] = 100 mM) under ambient conditions in 0.1 M NH_4OAc (pH 4.5).

Since aniline Schiff bases are not stable in water, the catalyst can be used at high concentrations, enabling large rate enhancements to be achieved. As a result, in the presence of aniline, mixtures of hydrazones can be rapidly equilibrated—within a day at room temperature at millimolar concentration without using a large excess of one of the reactants. In the absence of the aniline catalyst, the same equilibration would take weeks, greatly limiting its practical application in DCC. We anticipate this approach will facilitate the application of imine chemistry in dynamic materials and biological DCC applications.

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Supporting Information Available: Experimental details and the equations describing the reaction kinetics. This material is available free of charge via the Internet at http://pubs.acs.org.

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