General acid catalysis of the reversible addition of thiolate anions to cyanamide

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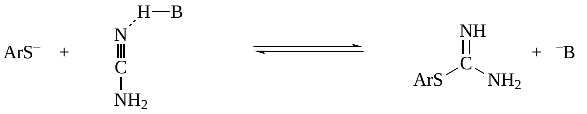
The addition of aromatic thiols (pK_a = 2.6–6.5) to cyanamide to give the isothiourea is subject to general-acid catalysis of the addition of thiolate anions by tertiary ammonium ions, with $\alpha = 0.26$ and 0.31 (±0.07) for the addition of thiophenol and 4-nitrothiophenol, respectively. The Brønsted coefficients and a small solvent isotope effect of $k_{H_2O}/k_{D_2O} = 1.6$ for catalysis by 4-morpholinoethanesulfonic acid (MES) buffer and for the uncatalysed addition of thiophenol are consistent with significant movement of a hydron in the transition state. The conclusion that the reaction proceeds through a basic asymmetric intermediate in the absence of catalysis is supported by the similar rate constants for the addition of thiophenolate anion to cyanamide and to *N*,*N*-dimethylcyanamide, which cannot tautomerize to the carbodiimide. The reaction has a central transition state in which S–C bond formation takes place with $\beta_{nuc} = 0.55$. It is concluded that the mechanism of catalysis involves partial transfer of a hydron to the cyano nitrogen atom in the transition state.

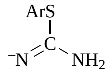
Introduction

In the course of an examination of the nucleophilic reactions of amines with 4-nitrophenylisothiouronium ion it became evident that some of the amine buffers were catalysing the expulsion of 4-nitrothiophenolate ion from the neutral isothiourea. A direct nucleophilic displacement of the thiolate anion from the isothiourea appeared unlikely, because although the corresponding nucleophilic displacement from the more electrophilic isothiouronium ion was observed with hydroxylamine it was not observed with any other amine. The catalysis appeared to be general-base catalysis which was surprising because it had been concluded in a previous study that the hydrolysis of phenylisothiourea proceeds by a reversible E1cB mechanism,[1] in which catalysis by proton transfer in the transition state is not kinetically significant. Examination of the reaction more closely in the reverse direction presents an interesting mechanistic ambiguity, because thiolate anions may add to either cyanamide or the more unstable tautomer carbodiimide to give the isothiourea. To determine which pathway is favoured and the mechanism of catalysis we have examined the reaction of thiolate anions and cyanamide in some detail.

We report here that the addition of substituted thiophenols to 0.33 M cyanamide is catalysed by amine buffers. The results are consistent with general-acid catalysis of the addition of the thiolate anion to cyanamide by protonated amines through a class e-mechanism,[2] which involves proton transfer to or from the electrophile and provides a facile pathway for addition–elimination reactions of many nucleophiles (Scheme 1). The results are consistent with the addition of thiolate anions to cyanamide (Scheme 1), rather than to the more reactive carbodiimide tautomer (HN=C=NH), in a stepwise mechanism that proceeds through the asymmetric intermediate, **1**. There is significant S–C bond formation, with $\beta_{nuc} = 0.55$, and partial transfer of a proton to the developing nitrogen anion from general acids or water in the transition state, with α ~0.25 and $k_{H_2O}/k_{D_2O} = 1.6$.



Scheme 1



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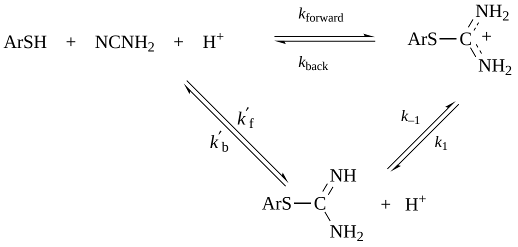
Results

Thiol addition and elimination

The reactions of thiophenols with cyanamide are complex because the reactions approach equilibrium and involve the acid dissociation of the thiol at low pH and the isothiouronium ion at high pH (Figs. 1 and 2). The slow formation of the isothiourea from thiol and cyanamide with the rate constant k'_f is followed by rapid proton transfer, k_{-1}, to give the isothiouronium ion (Scheme 2). This proton transfer provides the thermodynamic driving force for the synthesis, which becomes less favourable as the pK_a of the thiol decreases. Cleavage to the thiol and cyanamide becomes faster than formation of the isothiouronium ion at ~pH 5.3, 6.8 and 8.5 for pentafluorothiophenol (pK_a = 2.6), 4-nitrothiophenol (pK_a = 4.4) and thiophenol (pK_a = 6.2), respectively (Figs. 1 and 2). In the reverse direction rapid proton removal, k_1, is followed by slow expulsion of thiol from the isothiourea, with the rate constant k'_b, to give cyanamide (Scheme 2).



Scheme 2

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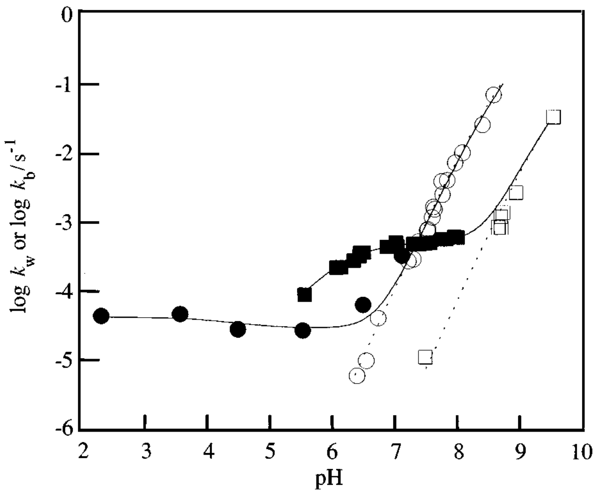

Fig. 1 Dependence on pH of the pseudo-first-order rate constants, k_w, for the approach to equilibrium of the reaction between 0.33 M cyanamide and thiophenol (■) or 4-nitrothiophenol (●) and the dependence on pH of the pseudo-first-order rate constants, k_b, for the disappearance of phenylisothiouronium ion (□) and 4-nitrophenylisothiouronium ion (○) at 40 °C, 1 mM EDTA and ionic strength 1.0 M (KCl). The dotted lines represent the best non-linear least squares fit according to eqn. (3) for the disappearance of the isothiouronium ions. The observed pseudo-first-order rate constants, k_w, for approach to equilibrium upon addition of the thiols to 0.33 M cyanamide (solid points) follow the rate law of eqn. (5). The solid lines represent the best fit according to eqn. (5) for the addition of the thiols to 0.33 M cyanamide and are extrapolated to high pH through the open data points, using the parameters previously determined for k_b. The parameters obtained from these fits are in Table 1.

Conditions were chosen such that the reactions were pseudo-first-order and followed the relationship, $k_w = k_f + k_b$.‡ The pseudo-first-order rate constants, k_f, for formation of the isothiouronium ion were found to be proportional to the concentration of cyanamide and the first-order rate constant, k_b, for cleavage of the isothiouronium ion is independent of cyanamide concentration over the range 0–0.33 M, as shown in the rate law of eqns. (1) and (2), respectively.

$$k_f[RSH][NCNH_2] = (k_0 + k_H[H^+])[RS^-][NCNH_2] \qquad (1)$$

$$k_b[\text{isothiouronium ion}] = (k'_{OH}[OH^-]^2 + k_2[OH^-])[\text{isothiouronium ion}] \qquad (2)$$

The rate constants for the formation, k_f, and cleavage, k_b, of isothiouronium ions were obtained from the buffer-independent rate constants, k_w, for approach to equilibrium. The pH rate profiles for the reaction between thiols and cyanamide display several distinct regions that are represented by the different terms in eqns. (1) and (2) and may be interpreted as described below.

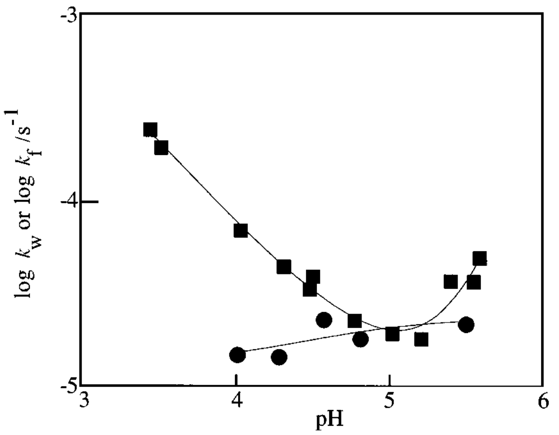

Fig. 2 Dependence on pH of the pseudo-first-order rate constants for the reactions of pentafluorothiophenol (■) and 3-nitrothiophenol (●), with 0.33 M cyanamide at 40 °C, 1 mM EDTA and ionic strength 1.0 M (KCl). The solid lines represent the best non-linear least squares fit of the rate constants for approach to equilibrium, $k_w = k_f + k_b$, for the reaction in the presence of 0.33 M cyanamide according to eqn. (5), where $[H^+] > K_1$ for pentafluorothiophenol and according to eqn. (4) for 3-nitrothiophenol.

The pH-independent region below pH 3.5 for the addition of 4-nitrothiophenol to 0.33 M cyanamide (Fig. 1) corresponds to the term $k_H[H^+]$ in eqn. (1), and presumably represents proton-catalysed addition of thiolate anion to cyanamide, although direct addition of the thiol cannot be excluded; in Fig. 1 this is represented by a slightly curved line which becomes significant below pH 4.4 where ionization of the thiol occurs. The reaction up to pH 6.5 corresponds to the uncatalysed addition of the thiolate anion to cyanamide, k_0 in eqn. (1), which follows the ionization of the thiol and is dominant until k_b becomes significant. Above pH 6.5 the addition of 4-nitrothiophenol is less than 100% and the increase in k_w corresponds to the rate constant, k_b, for the reverse reaction. The reverse reaction was also studied separately in the absence of cyanamide and between pH 6–8 the pH rate profile (Fig. 1) follows a slope of 2.0, corresponding to the term, $k'_{OH}[OH^-]^2$ in eqn. (2), under conditions in which the isothiourea is protonated. Above pH 8 the slope begins to decrease toward a slope of 1.0, corresponding to the acid dissociation of the isothiouronium ion (pK_a ~8.5, Table 1). Between pH 5.5–7.5, the pH rate profile for the addition of thiophenol to 0.33 M cyanamide (Fig. 1) follows the ionization of the thiol, corresponding to the term k_0 in eqn. (1). Above this pH the reverse reaction, k_b, becomes significant. The pH rate profile for the elimination of thiophenol (Fig. 1) follows a slope of 2.0 and decreases to a slope of 1.0 at pH 9.3, corresponding to the acid dissociation of the isothiouronium ion ($pK_a = 9.3$). The data in Fig. 1 do not extend to a high enough pH for the change in slope at pH 9.3 to be definitive, but it has been demonstrated previously.[1] The rate constant for the addition of pentafluorothiophenol to 0.33 M cyanamide (Fig. 2, ■) between pH 3.5–5 corresponds to the term $k_H[H^+]$ in eqn. (1) for the acid-catalysed addition of thiolate anion to cyanamide. There is a small pH-independent region centred at pH 5 that corresponds to k_0, the first term in eqn. (1) and above this pH the reverse reaction, k_b, becomes significant. The region between pH 4–5 in the pH rate profile for the addition of 3-nitrothiophenol to 0.33 M cyanamide (Fig. 2, ●) corresponds to the acid-catalysed addition of the thiolate anion to cyanamide and follows the ionization of the thiol. Above pH 5 the reaction corresponds to k_0. Elimination through a transition state with no net charge, k_2, is not significant over the pH range at which the rate constants were measured.

The observed rate constants for elimination of thiophenols from the isothiouronium ion, follow the rate law of eqn. (3) and

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‡ The rate constants used within this paper are defined thus: k_{obs} is the observed rate constant; $k'_{obs} = (k_{obs}/[NCNH_2])/(\text{fraction of } RS^-)$; k_w is the buffer-independent rate constant; k_0 is the second-order rate constant for addition of a thiolate anion to cyanamide; k_{OH} is the second-order rate constant for elimination of thiophenolate anion from an isothiourea catalysed by hydroxide ion; k_2 is the second-order and k'_{OH} is the third-order rate constant for elimination of thiophenolate anion from an isothiouronium ion catalysed by hydroxide ion, where $k_b = (k'_{OH}[OH^-]^2 + k_2[OH^-])$, from eqn. (2); k_H is the third-order rate constant for the proton-catalysed addition of a thiolate anion to cyanamide; k_f is defined in eqns. (1) and (4) and k_b is defined in eqns. (2) and (3). K_1 is the acid dissociation constant of an isothiouronium ion; k^f_{cat} is the rate constant for catalysis by the buffer of thiolate anion addition to cyanamide; k^b_{cat} is the rate constant for catalysis by the buffer of thiolate anion elimination from cyanamide; k_{HA} is the third-order rate constant for general-acid catalysis of the addition of thiolate ion to cyanamide; k_B, is the second-order rate constant for general-base catalysis of the elimination of thiolate ion from an isothiourea. $K_w = 4.016 \times 10^{-14}$ is the ionization constant of water[3] under the conditions of the kinetic experiments.

Table 1 Equilibrium and rate constants for addition and elimination reactions of thiolate anions with cyanamide at 40 °C and ionic strength 1.0 M (KCl)

Thiol	pK_a [a]	k_H [b]/M^{-2} s^{-1}	k_0 [c]/M^{-1} s^{-1}	k_{OH} [d]/M^{-1} s^{-1}	K_1 [e]/M	k_{OH} K_1 [f]/s^{-1}
2,3,4,5,6-F_5–ArSH	2.6	2.2	3.3×10^{-5} [g]			5.9×10^{-3}
4-NO_2–ArSH	4.4	2.95	8.7×10^{-5} [g]	6.7×10^{3} [f]	$3.6 \pm 2.5 \times 10^{-9}$ [f]	2.4×10^{-5}
3-NO_2–ArSH	4.5	1.5	7.2×10^{-5} [g]			
4-Br–ArSH	5.8		$8.2 \pm 0.1 \times 10^{-4}$ [h]			
4-F–ArSH	6.1		$1.8 \pm 0.1 \times 10^{-3}$ [h]			
PhSH	6.2		1.64×10^{-3} [g]	370 [i]	5.0×10^{-10} [j]	1.85×10^{-7}
3,5-Me_2–ArSH	6.5		$2.3 \pm 0.2 \times 10^{-3}$ [h]			
Thioglycolate	10.3 [k]		1.3 [h]			

[a] This work, measured as described previously.[22] [b] Third-order rate constant for specific-acid catalysis of the addition reaction. [c] Second-order rate constants for addition of RS^-. [d] Second-order rate constant for elimination of the thiolate anion from the isothiourea catalysed by hydroxide ion. [e] Acid dissociation constant of the isothiouronium ion. [f] Calculated from a fit to eqn. (3). [g] Estimated from a non-linear least squares fit of k_w *vs.* pH according to eqn. (5). [h] Estimated from an extrapolation to zero concentration of MES buffer, 50% free base, at pH 6.16. At this pH, $k_w = k_f = k_0[K_{ArSH}/(K_{ArSH} + H^+)]$ for 4-Br, 4-F and 3,5-Me substituted thiols. [i] Calculated from eqn. (3) and the previously determined[1] value of $K_1 = 5.0 \times 10^{-10}$ M. The effect of the 10 °C difference in temperature between this study and the temperature at which K_1 was measured previously is expected to be within the experimental error of the non-linear least squares fit, Fig. 1. [j] From ref. 1. [k] At 25 °C, from ref. 22.

$$k_b = \frac{k_{OH}K_1K_w/[H^+]}{[H^+] + K_1} \qquad (3)$$

were studied in the absence of cyanamide (open points, Fig. 1). The kinetic parameters in eqn. (3) were obtained from a non-linear fit of eqn. (3) to the pH rate profiles (dotted lines). Inclusion in eqn. (3) of the acid dissociation constant of the isothiouronium ion K_1, improves the fit for the elimination of 4-nitrothiophenol and thiophenol. A term corresponding to elimination through the neutral transition state is not included in eqn. (3) because it is not significant over the pH range that the elimination reactions were measured.

The rate constants for addition of the thiols to cyanamide (Fig. 1) follow the rate law of eqn. (4) at low pH; however at

$$k_f = \frac{K_{ArSH}[NCNH_2]}{K_{ArSH} + [H^+]}(k_H[H^+] + k_0) \qquad (4)$$

higher pH the rate constant, k_b, for the reverse reaction becomes kinetically significant. The rate constants for approach to equilibrium, k_w, for addition of the thiols to 0.33 M cyanamide (solid points) follow the rate law of eqn. (5), which is

$$k_w = \frac{K_{ArSH}[NCNH_2]}{K_{ArSH} + [H^+]}(k_H[H^+] + k_0) + \frac{k_{OH}K_1K_w/[H^+]}{[H^+] + K_1} \qquad (5)$$

obtained by combining eqns. (3) and (4) using the relationship $k_w = k_f + k_b$. In the presence of 0.33 M cyanamide elimination through a neutral transition state is not kinetically significant and therefore the corresponding term is not included in eqn. (5). The solid lines represent the best fit according to eqn. (5) for the addition of the thiols to 0.33 M cyanamide and is extrapolated to high pH through the open data points, using the parameters previously determined from the fit of the open data points in Fig. 1 to eqn. (3). The solid lines in Fig. 2 were determined according to eqn. (5) when $[H^+] > K_1$ for pentafluorothiophenol, and according to eqn. (4) between pH 4 and 5.5, for 3-nitrothiophenol where elimination of the thiophenol, k_b, is not significant. The parameters obtained from non-linear least squares analysis of the pH-rate profiles in Figs. 1 and 2, according to eqn. (5), are shown in Table 1, along with values of k_0 for reaction of the anions of 4-Br-, 4-F- and 3,5-dimethyl-thiophenol and for thioglycolate dianion. These rate constants were determined in MES § buffer (50% free base, pH 6.16) by extrapolating to zero buffer concentration. Under these conditions the reactions follow $k_w = k_f = k_0[K_{ArSH}/(K_{ArSH} + H^+)]$ for these thiols.

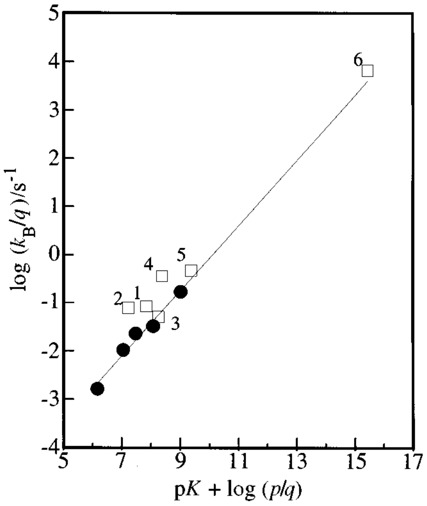

Fig. 3 Brønsted plot for the dependence of log (k_B/q) for general-base catalysis of the disappearance of 4-nitrothiophenylisothiourea on the pK + log (p/q) of the conjugate acid of the catalyst at 40 °C, 1 mM EDTA and ionic strength 1.0 M (KCl). The solid line with slope ($\beta = 0.67$) is the best linear fit to the Brønsted equation for the zwitterionic tertiary ammonium ions (●) and the open data points (□) represent; 1, EDA·H^+; 2, imidazole; 3, glycine amide; 4, TMPDA·H^+; 5, PDA·H^+; 6, hydroxide ion (Table 5).

Several lines of evidence indicate that the reaction is reversible and that nucleophilic substitution of the isothiouronium ion by hydroxide ion, the solvent or tertiary amines is not significant under the conditions of the experiments.

(*i*) In MOPS buffer, 20% base and 0.33 M cyanamide, k_b approaches k_f and a stable end point for the addition of 4-nitrothiophenol to cyanamide at equilibrium is observed at approximately 75% conversion to product. Nucleophilic substitution of the isothiouronium ion by hydroxide ion or the solvent would result in steady depletion of the cyanamide and biphasic kinetics, which were not observed.

(*ii*) The rate constant for catalysis by hydroxide ion shows little deviation from an extrapolation of the Brønsted correlation with $\beta = 0.67$ for general-base catalysis of the cleavage of 4-nitrophenylisothiourea by tertiary amines (Fig. 3). A different reaction mechanism for hydroxide ion would appear as a positive deviation from this line.

(*iii*) Cyanamide has been shown by Pratt and Bruice to be one of the products of the cleavage of phenylisothiourea at pH 8–10;[1] this can only be formed by abstraction of a proton in an elimination mechanism.

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§ Abbreviations: CHES, (2-cyclohexylamino)ethanesulfonic acid; MOPS, 4-morpholinopropanesulfonic acid; HEPES, N′-(2-hydroxyethyl)piperazine-N-ethanesulfonic acid; TAPS, 3-[tris(hydroxymethyl)-methylamino]-1-propanesulfonic acid; DTT, dithiothreitol; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); EDA, ethylenediamine; GA, glycine amide; Im, imidazole; TMPDA, tetramethylpropanediamine; PDA, 1,3-propanediamine.

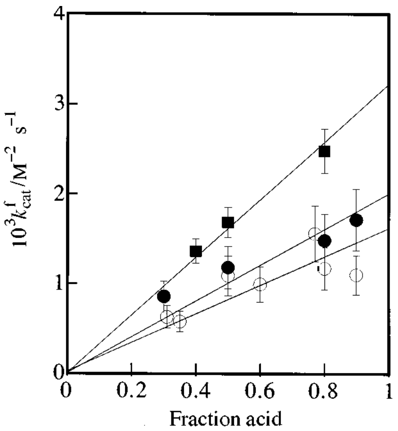


Fig. 4 Dependence of the third-order rate constants, k^f_{cat}, for general-acid catalysis of the addition of thiophenolate anion to cyanamide *versus* the fraction of the acid form of the catalysts HEPES (○), MOPS (●) and MES (■) at 40 °C, 1 mM EDTA and ionic strength 1.0 M (KCl). See Table 3 for buffer concentrations.

(*iv*) The fit of the data shown in Fig. 1 to the rate laws of eqns. (1) and (2) accounts for the dependence of the observed rate constants on pH without invoking an additional term for nucleophilic attack by hydroxide ion.

(*v*) The observed solvent isotope effect for catalysis of the addition of thiophenolate ion to cyanamide by MES acid suggests that proton transfer is significant in the transition state and that the reaction is subject to general-acid catalysis. The fact that the rate constant for catalysis by glycine amide falls on the Brønsted correlation for general-base catalysis by tertiary amines (Fig. 3) of the elimination of 4-nitrothiophenol from 4-nitrophenylisothiourea suggests that nucleophilic addition of glycine amide is not significant.

Catalysis of thiol addition

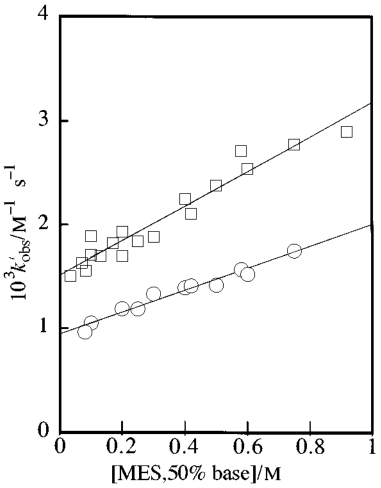


Fig. 5 Catalysis by MES buffer, 50% acid, of the addition of thiophenolate ion to cyanamide in H_2O (□) and D_2O (○) at 40 °C, 1 mM EDTA and ionic strength 1.0 M (KCl). The slopes of the lines correspond to $k_{HA} = (3.4 \pm 0.2) \times 10^{-3}$ and $(2.2 \pm 0.1) \times 10^{-3}$ M^{-2} s^{-1} for catalysis by the acid form of MES in H_2O and D_2O, respectively (Table 4), calculated assuming that general-base catalysis is not significant. Under the conditions of the reactions $k_w = k_f = k_0[K_{ArSH}/(K_{ArSH} + H^+)]$.

Catalysis by amine buffers of the addition to cyanamide of weakly basic aromatic thiols, with $pK_a = 2.6–6.5$, was observed to be weak but significant.[¶] Rate constants for general acid catalysis of the addition of thiophenolate ion by MES, MOPS and HEPES buffers at different fractions of buffer acid are shown in Fig. 4. These plots show scatter, because catalysis by the weaker catalysts (MOPS and HEPES) is difficult to measure at low fractions of acid. In 1.0 M HEPES buffer 31% acid, for example, catalysis accounts for 38% of the observed rate constant. A correction is made for a significant reverse reaction at high pH for MOPS and HEPES and this correction requires a knowledge of the dependence of the equilibrium[||] for the formation of the isothiourea on the pH of the solution and is dependent on $[H^+]^2$, which magnifies the uncertainty in the parameter. The error bars in Fig. 4 represent the degree of confidence in the data which are conservatively 10% for catalysis by MES and 20% for MOPS and HEPES. Most of the catalysis is observed under conditions in which most of the thiol is present as the anion so that it is unlikely that a significant fraction of the catalysis arises from general base catalysis of addition of the free thiol, which follows the same rate law as general acid catalysis of the addition of the thiolate anion. The correlation in Fig. 4 is forced through the origin of the plot because general-base catalysis would require the catalysis of the removal of a proton and it is not readily apparent which proton would be removed to facilitate the addition of a thiolate anion to cyanamide. Fig. 5 shows that there is a small solvent deuterium isotope effect of $k_{H_2O}/k_{D_2O} = 1.6 \pm 0.2$ for the uncatalysed reaction and for catalysis of the addition of thiophenolate ion to cyanamide by MES buffer, 50% free base, which is consistent with significant proton transfer in the rate-limiting transition state. Uncertainty in the fraction of ionized thiolate is not expected to affect the value significantly.

The addition of several thiols was studied with a series of catalysts in the pH range 2–9. The instability of the aryliso-thiouronium ion at high pH limited experiments at pH > 6 to thiols with $pK_a > 4$. General-acid catalysis was observed for the addition to cyanamide of the anion of 4-nitrothiophenol ($pK_a = 4.4$) by tertiary ammonium ions with pK_a 4.6–9.0 and for addition to cyanamide of the anion of thiophenol ($pK_a = 6.2$) by tertiary ammonium ions with pK_a 6.2–9.0. Rate constants for general-acid catalysis are reported in Tables 2–4. Catalysis by MES was examined for all of the thiols that were studied. No catalysis was detected with buffers of the uncharged formic acid and cyanoacetic acid.

The rate of addition of thioglycolate, a less acidic thiol, is slow at low pH, so that catalysis of the addition of thioglycolate by MES buffer, 50% base, was examined by the method of initial rates in two experiments. Fig. 6 shows that there is no catalysis by MES buffer, indicating that catalysis of the addition of thioglycolate to cyanamide by ammonium ions with $pK_a \geqslant 6$ is not significant. Aromatic thiolates are difficult to monitor by this method because they are prone to oxidation, especially at low pH where the co-ordination of metal ions by EDTA is less efficient; consequently the reactions of these thiols with cyanamide were not studied at pH values significantly below their pK_a.

The reaction rate for the addition of thiophenolate anion to 1,1-dimethylcyanamide was found to be only four-fold slower than that for addition to cyanamide and the reaction is catalysed by MES buffer to a similar extent (Table 4).

[¶] The rate of addition of thiophenol to cyanamide increases two-fold in the presence of 1 M MES buffer, 50% free base. When the catalysis is weak and the concentration of buffer is low, there is a danger that solvent effects may be significant.[4] In the present study plots of rate constants against buffer concentration are linear for all of the buffers. Many of the buffers are structurally related so that any solvent or salt effects should not seriously affect comparisons. Although we do not claim a high degree of accuracy for the individual rate constants for catalysis by buffers in Tables 2–5, the Brønsted coefficients and solvent isotope effects are expected to be reliable because salt and solvent effects should be comparable for the different catalysts.

[||] The equilibrium constant, $K = k_f/k_b$, for the addition of thiols to cyanamide was determined using eqns. (3) and (4) and the parameters in Table 1. A calculation of the equilibrium constant for the addition of 4-nitrothiophenol to 0.33 M cyanamide at pH 6.45 in MOPS buffer, 20% base of $K = 11.5$ M^{-1} is in satisfactory agreement with the observed value of $K = 9$ M^{-1}, that was measured by absorbance.

Table 2 General-acid catalysis of the addition of 4-nitrothiophenolate anion to cyanamide[a]

Catalyst	Concentration range/M	pK_a[b]	Fraction acid	$k^{\mathrm{f}}_{\mathrm{cat}}$[c]/M^{-2} s^{-1}	k_{HA}[d]/M^{-2} s^{-1}
Cyanoacetate	0.17–0.83	2.30	0.5	Not detectable[e]	
Formate	0.17–0.83	3.57	0.5	Not detectable[e]	
Dimethylpiperazine·H$^+$	0.08–0.42	4.63	0.5	2.7×10^{-4}	1.6×10^{-3}
MES	0.33–0.83	6.16	0.8	1.1×10^{-4}	1.3×10^{-4}
MOPS	0.17–0.83	7.05	0.8	1.1×10^{-4}	1.3×10^{-4}
CHES[f]	0.0–0.83	9.02	0.99	3.5×10^{-5}	3.5×10^{-5}

[a] Experimental conditions: 0.33 M cyanamide and 1 mM EDTA at 40 °C and ionic strength 1.0 M (KCl). [b] Calculated from pH measurements of the buffer solutions under the conditions of the kinetic experiments. [c] Third-order rate constant for buffer-catalysed addition of 4-nitrothiophenolate ion to cyanamide, corrected for the reverse reaction using the calculated equilibrium constant determined from the pH and the parameters in Table 1 and eqns. (3) and (4). [d] Third-order rate constants for general-acid catalysis of the addition to cyanamide. Obtained at one buffer ratio assuming that the observed catalysis is due to general-acid catalysis of the addition of the thiolate anion to cyanamide. [e] At the pH of the experiment the proton-catalysed reaction, k_{H}, dominates the rate equation [eqn. (1)]. [f] In 0.08 M MES buffer, 20% base.

Table 3 General-acid catalysis of the addition of thiophenolate anion to cyanamide[a]

Catalyst	Concentration range/M	pK_a[b]	Fraction acid	$k^{\mathrm{f}}_{\mathrm{cat}}$[c]/M^{-2} s^{-1}	k_{HA}[d]/10^{-4} M^{-2} s^{-1}
MES	0.17–0.83	6.16	0.8	25	32[e]
MES	0.03–1.0		0.5	17	
MES	0.07–0.20		0.4	14	
Phosphate	0.05–0.15	6.40	0.5	Not detectable	
Phosphate	0.05–0.15		0.2	Not detectable	
HEPES	0.17–0.50	7.48	0.9	11	16[e]
HEPES	0.17–0.83		0.8	12	
HEPES	0.17–0.83		0.6	9.9	
HEPES	0.17–0.83		0.5	11	
HEPES	0.17–0.83		0.35	5.7	
HEPES	0.17–0.83		0.31	6.2	
MOPS	0.08–0.42	7.05	0.9	17	20[e]
MOPS	0.17–0.83		0.8	15	
MOPS	0.17–0.83		0.5	12	
MOPS	0.17–0.83		0.3	8.5	
CHES[f]	0.0–0.83	9.02	0.99	5.8	5.8

[a] Experimental conditions: 0.33 M cyanamide and 1 mM EDTA at 40 °C and ionic strength 1.0 M (KCl). [b] Calculated from pH measurements of the buffer solutions under the conditions of the kinetic experiments. [c] Third-order rate constant for buffer-catalysed addition of thiophenolate anion to cyanamide corrected for the reverse reaction using the calculated equilibrium constant determined from the pH and the parameters in Table 1 and eqns. (3) and (4). [d] Third-order rate constants for general-acid catalysis of the addition to cyanamide. [e] Calculated from the intercept of the best fit of the data in Table 3 to a plot of $k^{\mathrm{f}}_{\mathrm{cat}}$ *vs.* the fraction of acid. The lines are drawn through 0 at fraction acid = 0 (Fig. 4). [f] In 0.08 M MES buffer, 20% base.

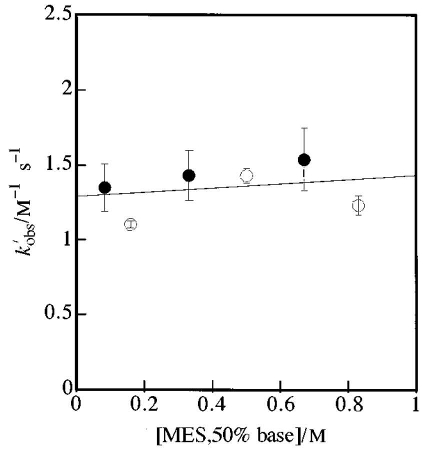


Fig. 6 Dependence of the second-order rate constants, k_{obs}, for the addition of thioglycolate dianion to cyanamide on the concentration of MES buffer, 50% base, at 40 °C, 1 mM EDTA and ionic strength 1.0 M (KCl). The solid and open circles represent two separate experiments. The fraction of thioglycolate dianion was determined from the pK_a of thioglycolate anion and the pH of the solution.

Catalysis of thiol elimination

It was possible to measure the rate of base-catalysed cleavage of the isothioureas in the absence of cyanamide, but buffer catalysis was difficult to measure. Corrections for small changes in pH are large because the rate of the buffer-independent elimination reaction depends on [H$^+$]^2 at the pH values at which the reaction was studied [eqn. (3)]. The second-order rate constants for general-base catalysis of the expulsion of 4-nitrothiophenolate ion from 4-nitrophenylisothiourea by several amines are given in Table 5.

Linear free energy relationships

Brønsted plots for general-acid catalysis of the addition of 4-nitrothiophenolate and thiophenolate ions to cyanamide follow slopes of $\alpha \sim 0.31$ and 0.26, respectively (Fig. 7). The rate constants for water (or hydroxide ion in Fig. 3) lie slightly above the extrapolated line through the catalytic constants for ammonium ions (amines in Fig. 3), while the rate constant for catalysis of the addition of 4-nitrothiophenolate ion by the proton deviates positively by an order of magnitude. The Brønsted slope of $\beta = 0.67 \pm 0.06$ for general-base catalysis of the elimination of 4-nitrothiophenolate ion in the reverse direction (Fig. 3) agrees well with the value of $\alpha = 0.31 \pm 0.07$ for general-acid catalysis of the reaction in the forward direction. Positive deviations of the rate constants for catalysis by monocations may arise from electrostatic interactions in the transition state. Although a nucleophilic mechanism has not been excluded for imidazole or ethylenediamine, glycine amide probably acts as a general-base catalyst because it falls on the Brønsted correlation for tertiary amines, which are expected to be relatively weak nucleophiles because of steric hindrance. Nucleophilic catalysis would provide an additional reaction pathway and would be expected to result in a positive deviation from this line, if it were significant.

Rate constants for the addition of substituted thiolate anions to cyanamide in water and catalysed by MES buffer (Tables 1

Table 4 Rate constants for the addition of substituted thiolate anions to cyanamide and dimethylcyanamide at 40 °C in water and catalysed by MES

Thiolate anion	pK_a	$k_0/10^{-4}$ M^{-1} s^{-1}	k_{HA}[a]/M^{-2} s^{-1}
2,3,4,5,6-F_5–PhS$^-$	2.6[b]		4.6 ± 1 × 10^{-5}[c]
4-NO_2–PhS$^-$	4.4[b]		1.3 ± 0.1 × 10^{-4}
3-NO_2–PhS$^-$	4.5[b]		2.7 ± 0.1 × 10^{-4}[c]
4-Br–PhS$^-$	5.8[b]		3.0 ± 0.8 × 10^{-3}[c]
4-F–PhS$^-$	6.1[b]		3.0 ± 0.4 × 10^{-3}[c]
PhS$^-$[d]	6.2[b]	15.0 ± 0.4	3.4 ± 0.2 × 10^{-3}
PhS$^-$ in D_2O[d]	6.5[b]	9.4 ± 0.3	2.2 ± 0.1 × 10^{-3}[c]
PhS$^-$ with $NCNMe_2$		4.0 ± 0.2	8.0 ± 0.2 × 10^{-4}[c]
3,5-Me_2–PhS$^-$	6.5[b]		3.8 ± 0.9 × 10^{-3}[c]
Thioglycolate	10.3[e]		Not detectable[f]

[a] Third-order rate constants for catalysis of the addition of thiolate anions by MES acid. [b] pK_a of the thiol at 40 °C, measured according to the procedure of Jencks and Salveson[22] with 1 mM EDTA and ionic strength 1.0 M (KCl). [c] Obtained in MES buffer (50% free base) assuming that the observed catalysis is due to general-acid catalysis of the addition of the thiolate anion to cyanamide. [d] These experiments were carried out consecutively under identical conditions. [e] Taken from ref. 22 at 25 °C. [f] Within experimental error (see Fig. 6).

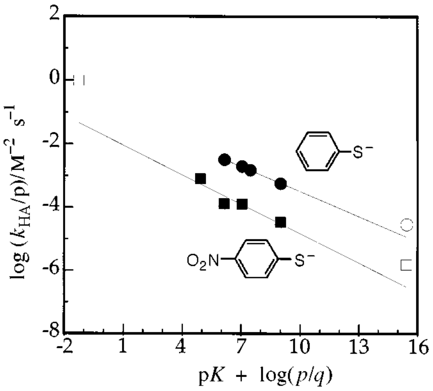


Fig. 7 Brønsted plot for the dependence of log (k_{HA}/p) for general-acid catalysis of the addition of 4-nitrothiophenolate anion (α = 0.31 ± 0.07, squares), (k_{HA} in Table 2) and thiophenolate anion (α = 0.26, circles), (k_{HA} in Table 3) to cyanamide on the pK + log (p/q), of the catalyst at 40 °C, 1mM EDTA and ionic strength 1.0 M (KCl). The solid lines represent the best linear fit to the Brønsted equation through the solid data points (●, ■). The open data points (○, □) represent either the proton or water.

and 4) follow a Brønsted correlation with a slope of β_{nuc} = 0.54 ± 0.07, as shown in Fig. 8.

Discussion

Tertiary ammonium ions catalyse the addition of substituted thiophenols to cyanamide to give the corresponding isothiourea by general-acid catalysis of the addition of the thiophenolate anion. The reaction is driven by protonation of the isothiourea to give an isothiouronium ion and is reversed at high pH values. The mechanism by which nucleophiles react with cyanamide has been uncertain because the tautomer, carbodiimide, may be present in solution at a low concentration[5,6] and could be the reactive species. Carbodiimides are known to react with many different nucleophiles[7] and appear to be significantly more reactive than cyanamide.**

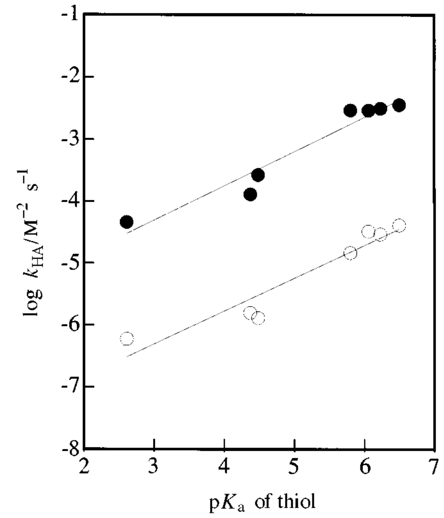
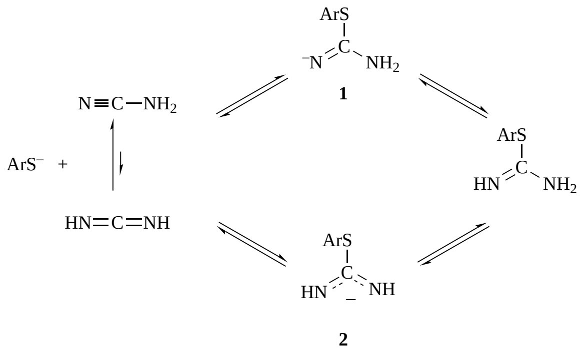


Fig. 8 Dependence on the pK_a of the thiol of the third-order rate constants for the addition of arylthiolate anions to cyanamide in water with β_{nuc} = 0.54 (obtained by dividing k_0 in Table 1 by the molarity of water) (○), or catalysed by MES acid with β_{nuc} = 0.55 (k_{HA} in Table 4) (●), at 40 °C and ionic strength 1.0 M (KCl)



Scheme 3

The formation of isothioureas from cyanamide and thiols could occur by one of two pathways: either by addition of the thiolate anion to cyanamide (N≡CNH_2) to give the unsymmetrical intermediate, **1**, or by addition to the more reactive, but less abundant tautomer, carbodiimide (HN=C=NH), to give the symmetrical intermediate **2** (Scheme 3). The pK_a of protonated **2** is expected to be well below that of protonated **1** because of the stabilizing effect of charge delocalization, so that it should be formed more readily by removal of a proton from the isothiourea product of the addition reaction. However, the data presented here indicate that although **2** is presumably more stable thermodynamically than **1**, the reaction mechanism proceeds through the more basic intermediate, **1** which is formed directly by the addition of ArS$^-$ to N≡CNH_2 and does not require proton transfer.

The similar rate constants for the addition of thiophenolate anion to cyanamide and to dimethylcyanamide, which cannot tautomerize (Table 4) support a common mechanism through the unsymmetrical ion, **1** rather than **2** (Scheme 3). The two reactions also show similar rate enhancements by MES buffer, 50% base (Table 4). The four-fold difference in rate may be attributed to a steric effect upon replacement of hydrogen atoms by methyl groups in *N*,*N*-dimethylcyanamide. The observation that expulsion of thiophenolate anion by hydroxide ion is 240-fold slower from phenyl 1,3-dimethylisothiourea than from isothiourea at 30 °C[1] provides further support for a mechanism that proceeds through **1** for the addition to cyanamide.

Proton transfer

The solvent isotope effect of k_{H_2O}/k_{D_2O} = 2.5 for general-acid catalysis of the addition of acetate anion to water-soluble carbodiimide provides evidence that the reaction is concerted: the

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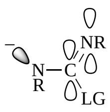
** It was not possible to assay cyanamide with two different assays that are used for carbodiimides because the reactivity of cyanamide in the assays is too low to measure.[8] No hydrolysis of cyanamide was detected over the time course of the kinetic runs, which in some cases was as much as 24 h. This may be compared with a water-soluble carbodiimide that has a half-life in water of 6 min at 25 °C.[9]

Table 5 General-base catalysis of the elimination of 4-nitrothiophenol from 4-nitrophenylisothiourea[a]

Catalyst[b]	pK_a[c]	Concentration range/M	k^{b}_{cat}[d]/M^{-1} s^{-1}	k_B[e]/M^{-1} s^{-1}
60% FB MES	6.16	0.067–0.40	0.001	1.6×10^{-3}
80% FB Im	6.93	0.167–0.83	0.062	7.8×10^{-2}
60% FB MOPS	7.05	0.067–0.40	0.006	1.0×10^{-2}
20% FB EDA·H$^+$	7.06	0.05–0.20	0.010	8.3×10^{-2}[f]
33% FB EDA·H$^+$		0.05–0.25	0.025	
50% FB EDA·H$^+$		0.067–0.20	0.053	
67% FB EDA·H$^+$		0.05–0.25	0.050	
10% FB HEPES	7.48	0.33–1.00	0.003	2.3×10^{-2}[f]
20% FB HEPES		0.33–1.00	0.010	
30% FB HEPES		0.33–1.00	0.009	
40% FB HEPES		0.33–1.00	0.012	
50% FB HEPES		0.33–1.00	0.011	
60% FB HEPES		0.13–0.40	0.012	
20% FB GA	7.78	0.083–0.42	0.013	5.1×10^{-2}[f]
40% FB GA		0.083–0.42	0.014	
50% FB GA		0.1–1.00	0.030	
60% FB GA		0.083–0.42	0.012	
50% FB PDA·H$^+$	8.63	0.067–0.20	0.23	4.7×10^{-1}
25% FB TAPS	8.09	0.13–0.40	0.008	3.3×10^{-2}
50% FB TMPDA·H$^+$	8.09	0.067–0.33	0.180	3.6×10^{-1}
50% FB CHES	9.02	0.13–0.40	0.085	1.7×10^{-1}

[a] Experimental conditions: 1 mM EDTA and ionic strength 1.0 M at 40 °C. [b] FB refers to the free base form of the buffer. [c] Calculated from pH measurements of the buffer solutions under the conditions of the kinetic experiments. [d] Second-order rate constants for buffer-catalysed elimination of 4-nitrothiophenolate anion. [e] Second-order rate constants for catalysis by the base form of the buffers. [f] Calculated from the intercept of the best fit through the data in Table 4 of a plot of k^{b}_{cat} *vs.* fraction of base. The lines are drawn through 0 at fraction base = 0 (Figure not shown).

proton is 'in flight' in the transition state.[9] It is possible that this concerted mechanism is enforced by the instability of the nitrogen anion, **3**, which may not be stabilized significantly by



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delocalization of the charge on the nitrogen atom into the neighbouring π system. The elimination of the thiophenolate anion from 1,3-dimethylisothiourea may also be concerted with proton transfer for the same reason, although the observed solvent deuterium isotope effect of $k_{H_2O}/k_{D_2O} = 0.72$ for this reaction was interpreted as evidence for an E1cB mechanism.[1] This does not, however, exclude a concerted mechanism that has a small primary solvent deuterium isotope effect with a significant secondary solvent deuterium isotope effect.

The small solvent isotope effect of $k_{H_2O}/k_{D_2O} = 1.6$ for general-acid catalysis of the addition of thiophenolate anion to cyanamide by MES, the Brønsted slope of $\alpha = 0.26$ and the small value of $p_{xy} \sim 0.03$†† are consistent with a mechanism in which catalysis occurs through hydrogen-bonding of the acid catalyst to the developing negative charge 'on' the sp-hybridized nitrogen atom of cyanamide in the transition state. Similar mechanisms in other systems, such as the addition of thiols to aldehydes,[10] exhibit comparable values of α. Water shows little deviation from the Brønsted correlation for general-acid catalysis by tertiary ammonium ions (Fig. 7). The small deuterium isotope effect of $k_{H_2O}/k_{D_2O} = 1.6$ is consistent with proton transfer in the rate-limiting step; a significant inverse secondary solvent isotope effect may contribute to this small isotope effect. A secondary isotope effect may arise from loss of solvation through hydrogen bonding of an LO$^-$ ion that participates in the abstraction of a proton from the isothiourea in the transition state.[13,14] In the addition direction this corresponds to a proton transfer from a water molecule to the developing nitrogen anion as the thiolate anion adds to the carbon atom of cyanamide.

The Brønsted α value provides a measure of the extent of proton transfer in the transition state of a reaction. A secondary solvent isotope effect for general-acid catalysis of the addition of thiophenolate anion to cyanamide of $(k_H/k_D)_{II} = 0.5^{-\alpha} = 1.2$ was estimated from the value of $\alpha = 0.26$ as described by Winey and Thornton.[15] This gives a calculated primary isotope effect, $(k_H/k_D)_I = 1.3$ from $(k_H/k_D)_I = (k_{H_2O}/k_{D_2O})/(k_H/k_D)_{II}$. The small primary isotope effect indicates that there is only a small loss of zero-point energy in the transition state and is consistent with catalysis by hydrogen bonding, or a concerted reaction in which coupling of heavy atom reorganization and proton transfer decreases the primary isotope effect.[16–18] The small isotope effect of $k_{H_2O}/k_{D_2O} = 1.6$ for catalysis by MES is probably closer to the true primary isotope effect for this process; a secondary isotope effect from a change in solvation of an ammonium ion is not expected to be significant.[14]

The rate constant for the addition of thioglycolate dianion to cyanamide is not significantly increased (<25%) by addition of 1 M MES buffer, 50% free base. This disappearance of catalysis of the addition of the more basic thiolate anion to cyanamide by MES buffer compared to the arylthiolate anions may result from a small decrease in α as the p*K* of the nucleophile increases or may be due to different properties of the alkyl and aromatic thiolate anions. The value of $p_{xy} = \partial\alpha/-\partial pK_{nuc} \sim 0.03$ obtained by comparing α for the addition of thiophenolate and 4-nitrothiophenolate anions to cyanamide would be enough to account for a decrease in α of >0.1 from thiophenol to thioglycolate and for the absence of significant catalysis by MES buffer for the addition of thioglycolate anion.

The rate constant for catalysis by H$^+$ deviates positively from the Brønsted plot for general-acid catalysis of the addition of 4-nitrothiophenolate anion to cyanamide by a factor of ~10-fold. This rate increase could arise from specific acid catalysis, which does not require movement of a proton in the transition state, or, possibly, from a cyclic transition state in which a water molecule mediates the transfer of a proton from the thiol to the nitrogen atom of cyanamide.

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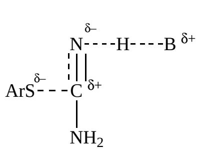
†† The variation of α with pK_{nuc} is defined by the structure–reactivity parameter, $p_{xy} = \partial\alpha/-\partial pK_{nuc} = \partial\beta_{nuc}/\partial pK_{HA}$.[11,12] A positive value of p_{xy} indicates that there is a significant interaction between the nucleophilic reagent and the proton donor in the transition state.

Concurrent stepwise and concerted mechanisms may occur when the formation of a reaction intermediate becomes particularly unfavourable. The pK_a of protonated cyanamide[19] is 1.0, so that the concentration of $HN{=}C{=}NH_2{}^+$, which is probably the thermodynamically stable tautomer, is very small near neutral pH. It appears that above pH 4 general-acid catalysis predominates. In a similar reaction, catalysis of the addition of acetate anion to a carbodiimide was judged to proceed *via* concurrent stepwise and concerted proton transfers.[9] In both cases protonation of the electrophile does not appear to increase greatly its reactivity towards the respective nucleophile. This may be the result of interactions between the nucleophile and the ‘soft’ polarizable electrophile at the transition state that are not effectively enhanced by protonation of the electrophile.

Structure of the transition state

The value of $\beta_{nuc} = 0.55$ indicates a central transition state for S–C bond formation in the addition of aromatic thiolate anions to cyanamide, in water and catalysed by the acid form of MES buffer.‡‡ The nitrogen atom undergoes a large increase in basicity as the intermediate anion is formed and this charge is stabilized by interaction with a proton donor with values of $\alpha \sim 0.26$ and of $p_{xy} = \partial\alpha/-\partial pK_{nuc} < 0.03$. A thermodynamically favourable interaction with water will occur if the nitrogen atom becomes more basic than hydroxide ion in the transition state. The observed solvent deuterium isotope effect and the fact that the rate constants for the reaction with water lie close to the extrapolated Brønsted lines support the conclusion that a proton of a water molecule interacts with the developing negative charge on the nitrogen atom in the transition state. Carbon–sulfur bond formation is *ca.* 50% complete at the transition state and it appears that the resulting charge development on the nitrogen atom is sufficient for a favourable hydrogen bonding interaction.



It is possible that the catalysis may occur through an enforced concerted mechanism, in which there is no significant barrier for expulsion of the thiolate anion from the anionic intermediate **1**. If the pK_a of the imino hydrogen of the isothiourea is 25,§§ the parameters in Table 1 and the relationship $k_e = k_{OH}K_w/K_a$ predict a rate constant of $k_e > 10^{13}$ s^{-1} for the elimination of PhS$^-$ from **1**. This value is comparable to the frequency of a C–S bond vibration and implies that the potential energy barrier for the expulsion of the thiolate anion may be insignificant.

Conclusions

The similar reactivities of dimethylcyanamide and cyanamide towards thiophenolate anion suggest that the reaction mechanism involves a transition state with a structure that approaches that of the asymmetric anion, **1**. The solvent isotope effects, the Brønsted correlations and the small value of p_{xy} are consistent with a stepwise mechanism ($\mathrm{A_{XH}D_H{}^{\ddagger *}D_N}$) of catalysis in which hydrogen bonding by general acids in the transition state provides catalysis of the addition of thiolate anion to cyanamide. However a concerted mechanism ($\mathrm{A_{XH}D_HD_N}$) that may be enforced by the lack of a significant barrier for the expulsion of the thiolate anion from the intermediate, **1**, or for proton transfer within a hydrogen-bonded intermediate species, $R_3NH^+\cdot{}^-N{=}C{-}NH_2$, in which the difference in pK_a between the catalyst and the intermediate may be >10 units cannot be excluded.

Experimental

Materials

Thiophenols were generally redistilled under nitrogen *in vacuo* before use. Cyanamide and potassium phosphate were the best commercially available grades and were used without further purification. The buffers, MES, CHES, MOPS, HEPES and TAPS, were obtained from Sigma and were used without further purification. 1,4-Dimethylpiperazine, ethylenediamine, glycine amide, tetramethylpropane-1,3-diamine and propane-1,3-diamine were recrystallized as their hydrochloride or dihydrochloride salts from aqueous ethanol. Imidazole was recrystallized from toluene. Formate and cyanoacetate were recrystallized from aqueous ethanol as their potassium salts. Millipore water containing 10^{-3} M disodium ethylenediaminetetraacetate (EDTA) was used throughout. Cyanamide was purchased from Aldrich and was stored frozen as a 4.0 M solution in 1.0 M KCl. The 99.99% isotopically pure D_2O used in this study was obtained from Cambridge Isotopes and DCl (99.99% isotopically pure) and KOD (98+% isotopically pure) from Aldrich, were titrated against a standard before use. Stock solutions of 4 M cyanamide for solvent isotope studies were prepared in H_2O or D_2O, lyophilized to dryness and redissolved in H_2O or D_2O containing 1 M KCl.

Phenyl- and 4-nitrophenyl-isothiourea were prepared following the method of Arndt[21] and showed ^{1}H NMR spectra in agreement with the proposed structures. The ionization constants of aromatic thiols were determined under the conditions of the kinetic measurements, as described previously.[22]

Kinetic measurements

Reaction solutions (with or without 0.33 M cyanamide) at ionic strength 1.0 M, maintained with KCl, were prepared in quartz cuvettes, purged with argon for several minutes and equilibrated to 40 °C before the addition of *ca.* 5 µl of a stock solution of 0.1 M thiol in acetonitrile or 0.1 M aqueous isothiourea at pH ~ 5. Following the addition, the solution was briefly purged, the top was tightly sealed and recording was commenced.

Several freshly prepared acidic thiols could not be stored in prepurged acetonitrile for more than a few hours before a precipitate formed, presumably the disulfide. To minimize contamination by disulfides a protocol was developed for 4-nitro, 3-nitro and 2,3,4,5,6-pentafluorothiophenol: a two-fold excess of sodium borohydride was added to a solution of the thiol in acetonitrile. After 5 min the mixture was rapidly filtered and a small aliquot (*ca.* 5 µl) of the filtrate was added to the reaction mixture in a 3000 µl quartz cell. The solution was then briefly purged, the top was tightly sealed and recording was commenced. This procedure gave consistent results. The oxidation of 2,3,4,5,6-pentafluorothiophenol at low pH was troublesome, but 10^{-4} M dithiothreitol was found to inhibit oxidation and permit satisfactory kinetic measurements.¶¶ The concentration of buffer was ⩾100 times that of the thiol (*ca.* 0.2 mM), so that pseudo-first-order conditions were maintained. Standard

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‡‡ A three point plot of log $k_{OH}K_1$ *vs.* pK_a of the thiol leaving group (Table 1) has a slope of −1.24. A value of $\alpha = 0.74$ for the dissociation of the isothiouronium ions (against the pK_a values of the thiols) would predict a value of $\beta_{lg} = -0.5$. This α value is observed for the dissociation of protonated alkyl benzimidates as a function of the pK_a of the alcohols[20] and may serve as an approximation of α for the reactions of isothioureas studied here. The values of $\beta_{nuc} = +0.55$ and $\beta_{lg} = -0.5$ (from $\beta_{eq} = \beta_{nuc} - \beta_{lg}$) indicates a central transition state for S–C bond formation.

§§ The pK_a values of a series of alkyl benzimidates ArOC(R)=NH have been estimated to be ~25.[20] This value is probably close to the pK_a values of the corresponding nitrogen acids of the isothioureas.

¶¶ The final concentration of sodium borohydride is <0.4 mM and therefore any reduction of cyanamide is insignificant. At this pH, DTT does not react with cyanamide at a significant rate.

conditions were 40 °C, 1 mM EDTA and ionic strength 1.0 M, maintained with potassium chloride.

Some reactions were followed in both directions. The addition of thiols to cyanamide was examined with 0.33 M cyanamide. Varying the cyanamide concentration over the range 0–0.33 M was found to have no significant effect on the rate constant for the elimination of thiophenol from phenylisothiourea. All pH measurements were made under the conditions of the kinetic experiments with an Orion Research Digital pH meter and a Corning 611 semi micro combination electrode. The pD of solutions in D_2O was measured using the same electrode, which had been standardized against (protium) standard buffers and was taken to be 0.4 units above the reading of the pH meter.[23]

The reactions with aromatic thiols were followed at the λ_{max} of the thiolate anion and the data were recorded on a computer attached to the spectrophotometer. The data were analysed with a Gateway 2000 4SX-33 computer using Sigma Plot. Reactions were generally followed for >5 half-lives and an end-point was taken after >10 half-lives. Rate constants were obtained from semilogarithmic plots of $(A_t - A_\infty)$ *vs.* time. These were linear for more than three half-lives for all the aromatic thiols studied and where duplicated the rate constants are generally to within 5–10%.

Rate constants for the addition of thioglycolate to cyanamide in MES buffer, 50% base, were obtained by the method of initial rates. The reactions were followed by monitoring the disappearance of thioglycolate by quenching aliquots of the reaction mixture with DTNB[24] and measuring the absorbance of the 4-nitrothiophenolate ion in 30 mM Tris buffer, pH 8.0. The end point for the reaction was assumed to correspond to the complete addition of the thioglycolate to cyanamide.

Rate constants for catalysis by buffers and for the uncatalysed reaction with water, k_w, were determined from the slopes and intercepts at zero buffer concentration, respectively, of plots of k_{obs} against the concentration of buffer.

The equilibrium constant K_{obs} for the addition of 4-nitrothiophenolate anion to 0.33 M cyanamide in MOPS buffer, 20% base was determined from the absorbance at the λ_{max} of the thiolate anion, 412 nm. The equilibrium constant was calculated using values of ε_{412} for the reactants and products that were determined separately.

Several substituted amines, such as ethylenediamine, were found to add directly to cyanamide with rate constants comparable to the rate constants for the addition of thiophenols and were not used as buffers or catalysts for the addition of thiols to cyanamide.

The solvent isotope effect for catalysis of the addition of thiophenolate anion to cyanamide by 0.05–1.0 M MES buffer, 50% base, was determined from rate constants obtained in H_2O and D_2O where thiophenol is 48 and 70% ionized, respectively.

Product analysis

The changes in the UV spectra for the reaction of aromatic thiols with cyanamide are consistent with the addition of the thiols to cyanamide to give an isothiouronium ion. The UV spectra of the products of the reactions of thiophenol and 4-nitrothiophenol with cyanamide are identical to those of phenylisothiourea and 4-nitrophenylisothiourea, respectively, in the presence of 0.33 M cyanamide and the changes of the spectra upon cleavage of these isothioureas at pH 8 are consistent with formation of the corresponding thiolate anions. It has been shown previously that phenylisothiourea is cleaved to cyanamide and thiophenolate anion at high pH.[1]

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