Kinetic study of the stability of $(NH_2)_2CSSC(NH_2)_2^{2+}$

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The kinetics of the decomposition of formamidine disulfide dihydrochloride, $(NH_2)_2CSSC(NH_2)_2^{2+}$ $2Cl^-$, in aqueous solution at 25 °C are general base catalysed, and rate constants have been measured over the pH range 1.91–9.03. Analysis of the data yields ionisation constants $pK_1 = 5.49$, $pK_2 = 7.66$ and rate constants for deprotonation of the substrate by OH^- and H_2O. For the doubly charged cation, reaction occurs through a small concentration of a reactive tautomer, $(HN{=})(NH_3^+)CSSC^+(NH_2)_2$, which is attacked by H_2O. The singly charged cation reacts by parallel pathways involving OH^- and H_2O as attacking bases.

The original work on the reaction of nitrous acid with thiourea by Storch[1] showed that a weak base was formed as a product in acidic solution. Detailed studies by A. E. Werner[2,3] established that the reaction involved a transient, red coloured intermediate, and that the weak base could be isolated as salts of formamidine disulfide, $(NH_2)_2CSSC(NH_2)_2^{2+}2X^-$, the *C,C′*-disulfanediylbis(dithioformamidinium) ion, reaction (1).

$$2H^+ + 2HNO_2 + 2(NH_2)_2CS \longrightarrow (NH_2)_2CSSC(NH_2)_2^{2+} + 2NO + 2H_2O \quad (1)$$

Werner found that in an excess of nitrous acid a slow further reaction took place, reaction (2), in which the formamidine disulfide was destroyed.

$$6HNO_2 + 3(NH_2)_2CSSC(NH_2)_2^{2+} \longrightarrow 6N_2 + 5HNCS + H_2SO_4 + HCN + 6H^+ \quad (2)$$

As part of our work on the chemistry of thionitrites we have identified[4] the red intermediate as $(NH_2)_2CSNO^+$, and have established[5,6] the kinetics and mechanism of reaction (1). We now describe our studies on the stability of the formamidine disulfide dication, $FDSH_2^{2+}$, as a prelude to our work on reaction (2). The structure of salts of formamidine disulfide have been established by Foss *et al.*, and his paper includes a useful summary of previous work on their chemistry.[7]

Experimental

Materials

Formamidine disulfide dihydrochloride was made by the method of Werner,[2] oxidising thiourea with sulfuryl chloride. It had mp 154–156 °C (lit.[2] 155 °C). All other chemicals were AnalaR materials, and were used without further purification. Stock solutions of $FDSH_2^{2+}2Cl^-$ were kept in excess perchloric acid to minimise decomposition, and were made up freshly from the solid salt each day.

Kinetic methods

Reactions that proceeded at moderate rates (at 25 °C) were monitored spectrophotometrically at 235 nm, using a UNICAM SP 8625. In more alkaline solutions the reaction was much faster and stopped-flow methods were necessary; here a solution of $FDSH_2^{2+}$ in an excess of perchloric acid (0.1 mol dm^{-3}) in one reservoir was mixed with an equal volume of buffer solution containing 0.1 mol dm^{-3} of sodium hydroxide to neutralise the perchloric acid from the other reservoir in a Hi Tech stopped-flow. Solutions were made up to a constant ionic strength of 0.5 mol dm^{-3} with $NaClO_4$. Experiments from pH 1.91 to 3.38 were buffered with an excess of perchloric acid, while from 4.06 to 5.39 acetate buffers were used. From pH 5.86 to 7.74 and from 8.25 to 9.03 phosphate and borate buffers respectively were used. All buffer concentrations were 0.05 mol dm^{-3}. For solutions in D_2O it was assumed that pD = pH + 0.4.

Results and discussion

An essential prerequisite to any investigation of reaction (2) was a study to establish the stability of $FDSH_2^{2+}$ in aqueous solution, and to measure the two pK_a values. Werner reported that there was slow decomposition of $FDSH_2^{2+}2Cl^-$ in aqueous solution, but that in the presence of NaOH a much more rapid reaction, reaction (3), occurred. We have observed

$$2OH^- + (NH_2)_2CSSC(NH_2)_2^{2+} \longrightarrow (NH_2)_2CS + S + NH_2CN + 2H_2O \quad (3)$$

that aqueous solutions of $FDSH_2^{2+}2Cl^-$ deposit a precipitate of sulfur on standing. We have confirmed the nature of the decomposition products, and titrations with NaOH, monitored by pH changes, confirmed the 2 : 1 stoichiometry. However, only one point of inflexion in the titration curve was observed. During the titration the solutions were cloudy due to precipitation of sulfur, and so the decomposition reaction made it impossible to deduce reliable pK_a values. An approximate figure of 5.1 for pK_1 was obtained by the rapid addition of half an equivalent of NaOH to aqueous $FDSH_2^{2+}$ and measurement of the pH as a function of time. The curve was extrapolated to estimate the pH at the time of mixing.

The kinetics of decomposition were followed in dilute solution, 2×10^{-5} mol dm^{-3}, and individual runs gave good first order plots yielding a measured rate constant k_o/s^{-1} which increased very strongly with increase in pH. For the very slowest runs, k_o values were obtained from initial rate measurements. A selection of results is listed in Table 1. Altogether, the reaction was studied at 41 pH values and we show a selection of 15 of these runs covering the full pH range studied together with a few values in D_2O solution. Table 1 also shows values of the rate constant predicted by our mechanism, k_o^*, at the same acidities. Measurements made at pH values

Table 1 Variation of k_o with acidity at 25 °C[a]

pH	$k_o/10^{-5}$ s^{-1}	$k_o^*/10^{-5}$ s^{-1}
1.91	3.58	3.43
2.02	3.98	4.03
2.43	9.15	8.24
3.38	64.9	62.3
4.12	319	327
4.22	446	410
4.93	1 720	1 790
5.26	3 350	3 210
5.39	4 160	3 940
6.09	10 300	10 200
6.89	33 000	33 200
7.34	60 500	66 300
8.25	144 000	153 000
8.83	182 000	179 000
9.03	187 000	183 000
pD		
1.75	0.42	
1.92	0.60	
2.15	1.00	
2.75	5.30	
3.04	9.89	

[a] k_o^* values calculated from parameters listed in the text by non-linear least squares fitting.

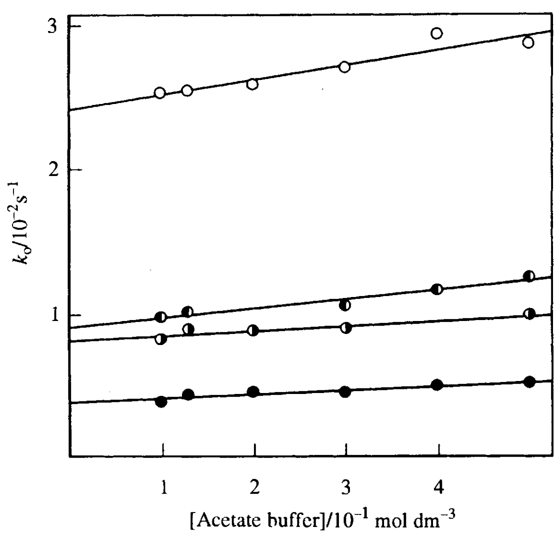
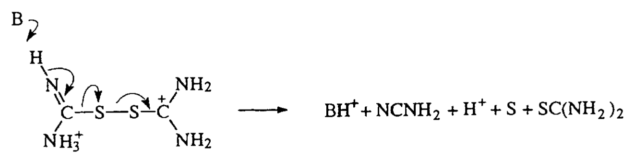


Fig. 1 Variation of k_o with concentration of acetate buffer. ○, pH 5.32; ◐, pH 4.90; ◑, pH 4.61; ●, pH 4.36.

greater than 3.4 used buffer solutions; at lower pH values the acidity was controlled by the concentration of an excess of perchloric acid. Checks on the effect of buffer concentration on k_o showed that there is a small amount of buffer catalysis, though the changes in k_o with [buffer] are very much smaller than those due to changes in pH. A plot of k_o as a function of acetate buffer concentration is shown in Fig. 1. The observation of a small amount of buffer catalysis could be due either to general base catalysis, or some form of nucleophilic catalysis. Addition of NaBr showed almost no effect on the rate, and as bromide ion is a very weak base but is a reasonably reactive nucleophile we have rejected nucleophilic catalysis as an explanation. However, it is well established[7] that sulfur nucleophiles can substitute at the disulfide bond in formamidine disulfide. We suggest that buffer catalysis is due to removal of a proton from $FDSH_2^{2+}$ or a tautomer. For reasons set out below we think that the latter is more likely, and the reaction is shown in Scheme 1.



Scheme 1

To analyse the variation of k_o with pH we concentrated initially on the results at the high and low acidity ends of our range of $[H^+]$. At low pH a plot of log k_o *versus* pH yielded a straight line of slope 0.86. Assuming that the major component is $(NH_2)_2CSSC(NH_2)_2^{2+}$, this suggests a reaction between $FDSH_2^{2+}$ and OH^- (or something kinetically equivalent). The fact that the order is substantially lower than unity could indicate a parallel pathway with H_2O acting as the base as shown in reaction (4). Writing the ionisation constants as K_1

$$FDSH_2^{2+} \begin{array}{c} \xrightarrow{k_1[OH]^-} \\ \xrightarrow[k_2[H_2O]]{} \end{array} \qquad (4)$$

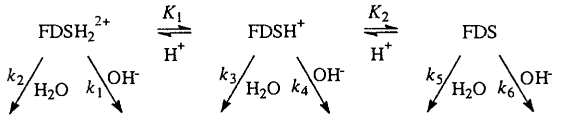
and K_2, and assuming only a small amount of ionisation at these acidities leads to the rate law shown in eqn. (5), and a plot

$$k_o = k_1K_w[H^+]^{-1} + k_2 \qquad (5)$$

of this equation from pH 1.91 to 2.66 yields $k_2 = 1.1 \times 10^{-5}$ s^{-1} and $k_1K_w = 3.0 \times 10^{-7}$, which gives $k_1 = 3.3 \times 10^6$ mol^{-1} dm^3 s^{-1}. The term $k_1[FDSH_2^{2+}][OH^-]$ could, of course, be replaced by the kinetically equivalent $(k_1K_w/K_1)[FDSH^+]$, corresponding to attack by H_2O on $FDSH^+$. At the high pH end of the scale it can be seen that k_o levels off. This suggests that the rate is proportional to the concentration of a free base species that is the major component of a conjugate acid–base pair. A reasonable mechanism for this is shown in reaction (6).

$$FDSH^+ \overset{K_2}{\rightleftharpoons} H^+ + FDS \xrightarrow[H_2O]{k} \text{products} \qquad (6)$$

This leads to the rate law $k_o = kK_1K_2/(K_1K_2 + K_1[H^+])$ on the assumption that there is a negligible proportion of $FDSH_2^{2+}$ at these pH values. A plot of $1/k_o$ *versus* $[H^+]$ yields a straight line with $K_2 = 2.2 \times 10^{-8}$ mol dm^{-3} and $k = 2$ s^{-1}. An alternative pathway that leads to an equivalent dependence upon acidity is attack by OH^- on $FDSH^+$, with a bimolecular rate constant of 2.2×10^6 mol^{-1} dm^3 s^{-1}. A general mechanism can be set out as shown below in Scheme 2.



Scheme 2

Because the k_o values level off around pH 9 it seems unlikely that the $k_6[OH^-]$ term contributes significantly to the rate at these acidities and the rate law may be written as in eqn. (7). This can be rearranged to eqn. (8). This can be expressed in the

$$k_o = \frac{k_5K_1K_2 + k_4K_wK_1 + (k_1K_w + k_3K_1)[H^+] + k_2[H^+]^2}{(K_1K_2 + K_1[H^+] + [H^+]^2)} \qquad (7)$$

$$k_o = \frac{1 + \dfrac{(k_1K_w + k_3K_1)[H^+]}{(k_5K_1K_2 + k_4K_wK_1)} + \dfrac{k_2[H^+]^2}{(k_5K_1K_2 + k_4K_wK_1)}}{\dfrac{K_2}{(k_5K_2 + k_4K_w)} + \dfrac{[H^+]}{(k_5K_2 + k_4K_w)} + \dfrac{[H^+]^2}{(k_5K_1K_2 + k_4K_wK_1)}} \qquad (8)$$

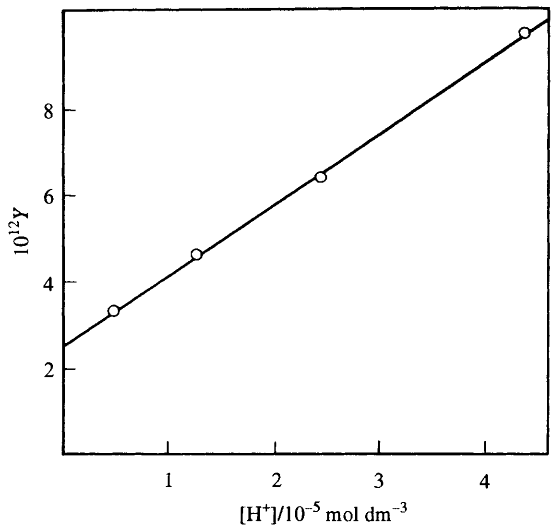


Fig. 2 Plot of acetate buffer data. $Y = S(K_1 + [H^+])(K_a + [H^+])$.

form $k_o = (1 + ax + bx^2)/(c + dx + ex^2)$ where $x = [H^+]$, and approximate values are available for K_2, k_4 and k_2. By means of a non-linear regression computer analysis the following values were obtained: $K_1 = (3.26 \pm 0.52) \times 10^{-6}$ mol dm^{-3}, $K_2 = (2.17 \pm 0.14) \times 10^{-8}$ mol dm^{-3}, $k_2 = (1.35 \pm 0.23) \times 10^{-5}$ s^{-1}, $(k_1K_w + k_3K_1) = (2.56 \pm 0.52) \times 10^{-7}$ mol dm^{-3} s^{-1}, $(k_4K_w + k_5K_2) = (4.15 \pm 0.22) \times 10^{-8}$ mol dm^{-3} s^{-1}. It will be noted that these values, based on fitting the full data set, are quite close to the approximate values obtained by plotting appropriate functions for the points at the highest and lowest pH values. The agreement between observed and calculated values of k_o is excellent. For a total of 41 points the mean deviation is 5.3% over a reactivity range of 10^5 and 7 units of pH. A selection of the data is shown in Table 1. The pK_1 value of 5.5 is distinctly higher than the value of 5.1 estimated by the direct measurement, but the uncertainties of extrapolation lead us to prefer the value obtained by the curve fitting procedure. As pK_1 and pK_2 differ by only two units there will be a significant amount of overlap in the ionisation ranges of the two protons in $FDSH_2^{2+}$.

With these values of K_1 and K_2 we can now analyse the buffer catalysed runs. Taking k_{B1} and k_{B2} as the rate constants (mol^{-1} dm^3 s^{-1}) for the reaction of the buffer anion with $FDSH_2^{2+}$ and $FDSH^+$ respectively, then the slope (S) of a plot of k_o *versus* [buffer] is given by eqn. (9), where K_a is the dissociation

$$S = \frac{(k_{B1}[H^+] + k_{B2}K_1)K_a}{(K_1 + [H^+])(K_a + [H^+])} \qquad (9)$$

constant of the buffer acid and values of k_{B1} and k_{B2} can be obtained by plotting eqn. (10). A plot for the acetate data is

$$S(K_1 + [H^+])(K_2 + [H^+]) = k_{B2}K_1K_a + k_{B1}K_a[H^+] \qquad (10)$$

shown in Fig. 2. We find the bimolecular rate constants $k_{B1}(CH_3COO^-) = 6.53 \times 10^{-3}$, $k_{B2}(CH_3COO^-) = 1.54 \times 10^{-2}$ and $k_{B1}(ClCH_2COO^-) = 5.95 \times 10^{-4}$. From the k_{B1} values we can calculate a value of 0.56 for the Bronsted β coefficient for general base catalysis of the decomposition of $FDSH^+$ suggesting partial transfer of the proton in the transition state. If we compare the k_{B1} and k_{B2} values for attack of acetate ion on $FDSH_2^{2+}$ and $FDSH^+$ we see that the former appears to be less reactive, even though it is the stronger acid by 2.17 units of pK_a. This can be understood if the reactive form is a tautomer, present in small amount, as shown in Scheme 1. If the proton is about half transferred, as suggested by the β value, then the α value for $FDSH_2^{2+}$ and $FDSH^+$ may also be around 0.5, and this would require a tautomerisation constant of around 10^{-5}. This is not a compelling argument, because the proton being transferred, probably the imino hydrogen, is not the same as that responsible for the acidity [presumably in the $-C(NH_2)_2^+$ group].

Table 2 Values of rate constants

$FDSH_2^{2+} \rightleftharpoons FDSH_2T^{2+}$	$K_T < 10^{-5.5}$
$OH^- + FDSH_2T^{2+} \longrightarrow$	$10^{10} \geqslant k_1^T \geqslant 4.15 \times 10^6$ mol^{-1} dm^3 s^{-1}
$(H_2O + FDSH_2^{2+}) \longrightarrow$	$k_2 = 1.35 \times 10^{-5}$ s^{-1}
$H_2O + FDSH_2T^{2+} \longrightarrow$	$k_2^T > 4.3$ s^{-1}
$H_2O + FDSH^+ \longrightarrow$	$k_3 \approx 0.08$ s^{-1}
$OH^- + FDSH^+ \longrightarrow$	$k_4 = 4.15 \times 10^6$ mol^{-1} dm^3 s^{-1}

Turning now to the analysis of the data, we need to consider the ambiguity that the terms k_5[FDS] and $k_4[FDSH^+][OH^-]$ lead to the same acidity dependence (as also do $k_1[FDSH_2^{2+}][OH^-]$ and $k_3[FDSH^+]$). If we assume that k_5K_2 is much greater than k_4K_w, then as $K_2 = 2.17 \times 10^{-8}$ it follows that $k_5 = 1.91$ s^{-1}. Now k_2 is 1.35×10^{-5} s^{-1}, and it seems most improbable that water would attack a neutral substrate FDS five orders of magnitude more rapidly than the doubly charged $FDSH_2^{2+}$. This could again be accounted for if the reactant was a tautomeric species such as $(HN{=})(NH_3^+)CSSC^+(NH_2)_2$, which we denote as $FDSH_2T^{2+}$; a tautomeric equilibrium constant in the range $K_T = [FDSH_2T^{2+}]/[FDSH_2^{2+}]$ in the range 10^{-5} to 10^{-6} gives a reactivity greater than 1.91 s^{-1}. If we take K_T as $10^{-5.5}$, then the rate constant for the reactive tautomer, $k_2^T = k_2/K_T = 4.3$ s^{-1}. Assuming that any hydroxide ion path also proceeds *via* the same tautomer with a rate constant k_1^T/mol^{-1} dm^3 s^{-1}, then the maximum value for k_1^T is 10^{10}, and $k_1 = k_1^TK_T$ is *ca.* $10^{4.5}$ mol^{-1} dm^3 s^{-1}. This requires that k_1K_w is *ca.* $10^{-9.5}$ mol dm^{-3} s^{-1} which means that $k_3K_1 \approx 2.5 \times 10^{-7}$ mol dm^{-3} s^{-1}. From our known value of K_1 this makes $k_3 \approx 0.08$ s^{-1}. The combination of reactivities of 4.3, 0.08 and 2.1 s^{-1} for $FDSH_2T^{2+}$, $FDSH^+$ and FDS does not seem very likely; the reactivity of $FDSH^+$ seems out of sequence, and it seems basically unlikely that neutral FDS should be so close in reactivity to the doubly charged tautomer of $FDSH_2^{2+}$. An even smaller value of K_T will increase the relative reactivity of $FDSH_2^+$ to FDS, but will still leave $FDSH^+$ out of sequence. An alternative assumption is that $k_5K_2 \ll k_4K_w$, which leads to $k_4 = 4.15 \times 10^6$ mol^{-1} dm^3 s^{-1}. To proceed further we must now consider the other ambiguity, the relative values of k_1K_w and k_3K_1. If we assume that $k_3K_1 \gg k_1K_w$, then $k_3K_1 = 2.56 \times 10^{-7}$ mol dm^{-3} s^{-1} and hence $k_3 = 0.079$ s^{-1}. It is reasonable to suppose that the active tautomer $FDSH_2T^{2+}$ will react more rapidly than $FDSH^+$, and that its rate constant must be greater than 0.079 s^{-1}. If we write $k_2 = k_2^TK_T$, then $K_T \leqslant 1.71 \times 10^{-4}$. Now k_1^T must be less than the encounter rate, 10^{10} mol^{-1} dm^3 s^{-1}, and as $k_1 = k_1^TK_T$, then this means that $k_1 \leqslant 1.7 \times 10^6$ mol^{-1} dm^3 s^{-1} and $k_1K_w \leqslant 1.7 \times 10^{-8}$ mol dm^{-3} s^{-1}. This is consistent with our assumption that $k_3K_1 \gg k_1K_w$. This enables us to set out the values in Table 2. These are possible values, though we are constrained by having only a limit for K_T. The reasonableness of this limit is discussed below. However, we first need to consider the other possible inequality, that $k_1K_w \gg k_3K_1$. This means that $k_1 = 2.56 \times 10^7$ mol^{-1} dm^3 s^{-1}. As $k_1 = k_1^TK_T$, and k_1^T must be less than or equal to 10^{10} mol^{-1} dm^3 s^{-1}, then $K_T \geqslant 2.56 \times 10^{-3}$. This seems surprisingly high. It implies that $k_2^T \leqslant 5.3 \times 10^{-3}$ s^{-1}. Again on the assumption that the tautomer of $FDSH_2^{2+}$ will be more reactive than $FDSH^+$ towards OH^-, we can conclude that $k_1^T > 4.15 \times 10^6$ mol^{-1} dm^3 s^{-1}. This implies that K_T is less than 6.17. While this seems certain to be true it represents an unrealistic upper limit. We conclude that the values deduced from the assumption that $k_1K_w \gg k_3K_1$ are improbable, and we prefer opposite inequality. The intermediate case $k_1K_w = k_3K_1$ leads to the unreasonable conclusion that $k_3 > k_2^T$, *i.e.* $FDSH^+$ is more reactive than the tautomer of $FDSH_2^{2+}$ towards OH^-.

The difficulty with this analysis lies in the assignment of a value for K_T, and it is necessary to consider if the figure $K_T \leqslant 10^{-5.5}$ is reasonable. The question is the relative basicity of the imino and amino groups in $FDSH^+$, $(NH_2)_2C^+$-$SSC(=NH)NH_2$. Stewart comments[8] that the amidino group certainly protonates at the imino nitrogen to give a resonance stabilised cation $RC^+(NH_2)_2$, and he draws attention to the analogy with the protonation of the carboxy group with forms $RC^+(OH)_2$. Charton has reviewed the ionisation constants of a range of amidinium cations.[9] In $NH_2C(=NH)(NH_2)$ and $CH_3C(=NH)(NH_2)$ protonation occurs on the imino group, and the methyl substitution only changes the pK_a of the conjugate acid by 1.2 units, from 13.6 to 12.4. The methyl compound is analogous to acetamide, $CH_3C(=O)(NH_2)$ and here again protonation occurs on the double-bonded atom. Fersht[10] has calculated that the relative basicities of the amino and carbonyl groups is *ca.* 10^{-7}. Stewart has commented[11] that $ROC(=NH)(NH_2)$ is about 10^{10} times as basic as $NH_2C(=O)NH_2$ which in any case protonates on the oxygen. Thus the relative basicity of the imino and amino groups must be even greater. With these comparisons, our estimate of $K_T \leqslant 10^{-5.5}$ seems reasonable.

We now turn to the solvent isotope effect data. The measurements in D_2O were restricted to the acidic end of our scale, from pD 1.75 to 3.04. At these acidities formamidine disulfide is more than 99% present as $FDSH_2^{2+}$, and the rate law approximates to eqn. (11). Our data did not provide a

$$k_o = k_2^T K_T + (k_1^T K_T K_w + k_3 K_1)[H^+]^{-1} \qquad (11)$$

reliable value for the intercept of a plot of k_o against $[H^+]^{-1}$, and so the solvent isotope effect on $k_2^T K_T$ remains unknown. The ratio of the slopes, H/D, was 3.05. To interpret this we need to distinguish between the effects on K_1 and k_3. If we take a lower limit for $(K_1)_H/(K_1)_D$ to be 2, then $(k_3)_H/(k_3)_D = 1.53$ corresponding to partial stretching of the bond. However, many substituted anilinium ions have $(K_a)_H/(K_a)_D$ values close to 3.5, and use of this as a model for $FDSH_2^{2+}$ would give $(k_3)_H/(k_3)_D = 0.86$. A treatment based upon fractionation factors requires $(k_3)_H/(k_3)_D = 1/(\varphi_1^2\varphi_2)$ where φ_1 and φ_2 are the factors for the proton of the water molecule acting as a base and the proton 'in flight' respectively. If φ_1 is written as $0.69^{1-\theta}$, where 0.69 is the fractionation factor for H_2O and θ is the slope of the Brønsted plot, then use of $(K_1)_H/(K_1)_D = 2$ leads to $\varphi_2 = 0.91$, within the acceptable range of 0.69 to 1. Use of 3.5 instead of 2 leads to $\varphi_2 = 1.6$. Thus the solvent isotope effect is consistent with our model provided that $(K_1)_H/(K_1)_D$ is around 2. A similar treatment on the assumption that k_1K_w is much greater than k_3K_1 leads to large discrepancies.

We conclude that the decomposition of formamidine disulfide proceeds mainly through the monoprotonated species $FDSH^+$, with parallel pathways involving proton removal by hydroxide ion and water. The doubly protonated species reacts through a tautomer by proton loss to water. There is no sign of a pathway involving attack by hydroxide ion on the tautomer, presumably because under acid conditions both species are present in very low concentrations. Thus at pH 2, $[OH^-] = 10^{-12}$ mol dm^{-3} and if $K_T \leqslant 10^{-5.5}$, then even if reaction occurred at the encounter rate, the value of k_o due to such a pathway would be $10^{10} \times 10^{-12} \times 10^{-5.5} = 10^{-7.5}$ s^{-1} two orders of magnitude less than the observed value of k_o. Thus reaction occurs by the much less basic, but omnipresent water. Formamidine disulfide has a well established role[12] in biochemistry as a potent inhibitor of the enzyme amidinase transferase. Its action is suggested to involve reaction at an enzyme sulfhydryl group to form enzyme–S–S–C(=NH)(NH$_2$), which blocks the use of the enzyme–SH function. With our values of K_1 and K_2 it can be calculated that at physiological pH of 7.4 formamidine disulfide contains 34.9% FDS, 64.3% $FDSH^+$ and 0.8% $FDSH_2^{2+}$. Certainly the presence of a large fraction of $FDSH^+$ could plausibly account for a reaction of the type shown in reaction (12).

$$\text{enzyme–SH} + (HN=)(NH_2)CSSC(NH_2)_2^+ \longrightarrow$$
$$\text{enzyme–S–S–C}(=NH)(NH_2) + H^+ + (NH_2)_2CS \qquad (12)$$

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