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Kinetics of the Cyanide–Cystine Reaction

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A spectrophotometric method was used to study the kinetics of the cystine–cyanide reaction in 0.04 *M* potassium hydroxide at *p*H 12.5. As expected for nucleophilic displacement, the reaction is bimolecular and cyclization of the thiocyanato product is much faster than the reverse reaction. Activation parameters at 35° for the reaction are: E_a, 16.8 kcal./mole; ΔH^*, 16.1 kcal./mole and ΔS^*, −7.4 e.u. The entropy of activation is of the same order of magnitude as is that for the reaction of CN^- with S_8 but some 20 e.u. less than that for the reaction of sulfite ion with cystine, indicating the activated complex for cystine–sulfite to be more crowded than that with cyanide ion.

Of the several mechanisms by which scission of a sulfur–sulfur bond occurs, nucleophilic displacement at a sulfur atom is of particular interest because of the variety of nucleophiles which may be used and the variety of products which may be obtained. As a typical nucleophilic displacement, the action of cyanide ion on various compounds containing a sulfur–sulfur bond has been studied.[1] These investigations have been concerned primarily with equilibrium aspects of the cyanide ion induced splitting. Kinetic investigations having been limited to the reaction of cyanide ion with sulfur,[2] S_8, and with thiosulfate[2] and in keeping with the nucleophilic displacement concept[3,4] both of these reactions showed bimolecular kinetics.

This investigation was undertaken to provide additional kinetic information on the reaction of cyanide ion with the sulfur–sulfur bond and in particular on the reaction of cyanide ion with disulfides. Cystine was chosen for study because the stoichiometry of the reaction is known and the reaction goes to completion due to cyclization of the product, S-cyanocysteine, to 2-aminothiazoline-4-carboxylic acid.[5,6] Cystine was also chosen because the reaction of cyanide ion with proteins is of general interest[7,8] and because splitting of the disulfide bond of cystine is pertinent to the biochemically important sulfhydryl–disulfide exchange reaction.[9] The kinetics of the reaction of cyanide with cystine also have been studied previously[10] in the presence of excess cyanide and the reaction was found to be first order with respect to appearance of cysteine.

Experimental

Materials.—Fisher AR potassium cyanide from freshly opened bottles was used. Fresh solutions were prepared prior to each run and were assayed with silver nitrate.[11]

Reagent grade cysteine hydrochloride monohydrate and cystine were purchased from Nutritional Biochemicals Corp. and used as such. Purity determinations by formol titration[12] and by amperometric titration[13] indicated the preparations to be 97.5% and 98.3% pure, respectively. A portion of an aqueous suspension of cystine was treated with hydrogen sulfide,[14] filtered, and washed with distilled water and dried *in vacuo*, at 100°, over phosphorus pentoxide. The cystine so treated assayed 100.2% by formol titration.

2-Amino-thiazoline-4-carboxylic acid was prepared by the procedure of Schöberl and Hamm[17] but the product was isolated from 70% alcohol as per Wood and Cooley.[18] Our product decomposed at 234–237° and exhibited $[\alpha]^{25}D$ −118°, both of these values being higher than those reported by Wood and Cooley. Cystine disulfoxide was prepared by the procedure of Toennies.[19]

Kinetic Procedure.—Kinetic runs were followed by spectrophotometric determination of appearance of product. At appropriate time intervals an aliquot of the reaction mixture was diluted 50 to several-hundred fold with 0.040 *M* potassium hydroxide. The dilution effectively stopped the reaction and the optical density of this solution at 235 mμ then was determined in a Beckman DU instrument. Optical density readings were converted to cysteine molarity by means of the equation

$$C_{RS} = \frac{O.D._t - O.D._0}{\epsilon_{RS^-} + \epsilon_{Th} - \epsilon_{RSSR} - \epsilon_{CN^-}} = \frac{O.D._t - O.D._0}{6.9 \times 10^{-3}}$$

The epsilons represent molar extinction coefficients, Th represents 2-amino-thiazoline-4-carboxylic acid and the other symbols have their usual meanings. The optical density at zero time was calculated from the molar extinction coefficients of cystine and cyanide and the initial concentrations of each reactant. The values of extinction coefficients employed, determined in 0.040 *M* potassium hydroxide, are: ϵ_{RS^-}, 4.97×10^3; ϵ_{Th}, 2.60×10^3; ϵ_{RSSR}, 0.417×10^3 and ϵ_{CN^-}, 0.250×10^3.

Reaction mixtures varying in concentration of reactants from 0.005 *M* to several-hundredths molar were made up by mixing at zero time equal volumes of cystine and cyanide solutions of appropriate concentration. Both cystine and cyanide solutions were prepared freshly prior to each run. Cystine solutions were prepared by dissolving the necessary quantity of cystine in the calculated quantity of 0.1 *M* base and diluting to 100 ml. with 0.040 *M* potassium hydroxide. Cyanide solutions were directly prepared in 0.040 *M* potassium hydroxide. The potassium hydroxide solution was thoroughly deoxygenated prior to use by passing purified nitrogen through the solution for 45 minutes. To minimize access of air to reaction mixtures during the run, the initial reaction mixture was apportioned into three glass-stoppered tubes, each tube being filled as completely as possible, and a given tube was used to follow the reaction for *ca.* one-third of the reaction time. Aliquots of the reaction mixture were

(1) A. J. Parker and N. Kharasch, *Chem. Revs.*, **59**, 583 (1959).
(2) P. D. Bartlett and R. E. Davis, *J. Am. Chem. Soc.*, **80**, 2513 (1958).
(3) O. Foss, *Acta Chem. Scand.*, **4**, 404 (1950).
(4) O. Foss, *Kgl. Norske Videnskab. Selskab. Skrifter*, **1945**, No. 2 (1947).
(5) A. Schöberl and M. Kawohl, *Angew. Chem.*, **64**, 643 (1952).
(6) A. Schöberl, M. Kawohl and R. Hamm, *Ber.*, **84**, 571 (1951).
(7) O. Gawron, J. Keil and A. J. Glaid, III, *Biochim. Biophys. Acta*, **19**, 170 (1956).
(8) W. R. Cuthbertson and H. Phillips, *Biochem. J.*, **39**, 7 (1945).
(9) For a recent review see E. V. Jensen, *Science*, **130**, 1319 (1959).
(10) T. Hata and S. Matsushita, *Mem. Res. Inst. Food Sci., Kyoto Univ.*, No. 9, 19 (1955).
(11) I. M. Kolthoff and E. B. Sandell, "Textbook of Quantitative Inorganic Analysis," The Macmillan Co., New York, N. Y., 1952, pp. 458–460.
(12) In the case of cystine, excess standard alkali and formaldehyde were added initially and a potentiometric titration with standard hydrochloric acid was carried out.
(13) I. M. Kolthoff and W. Stricks, *J. Am. Chem. Soc.*, **72**, 1952 (1950).
(14) This treatment which removes cystine disulfoxide[15] was carried out although cystine disulfoxide could not be detected analytically[16] in the cystine.
(15) B. Sorbo, *Biochim. Biophys. Acta*, **22**, 570 (1956).
(16) T. F. Lavine, *J. Biol. Chem.*, **113**, 584 (1936).
(17) A. Schöberl and R. Hamm, *Ber.*, **81**, 210 (1948).
(18) J. L. Wood and S. L. Cooley, *J. Biol. Chem.*, **218**, 449 (1956).
(19) G. Toennies, *ibid.*, **113**, 580 (1936). For a discussion of the structure of "cystine disulfoxide" see B. J. Sweetman, *Nature*, **183**, 744 (1959).

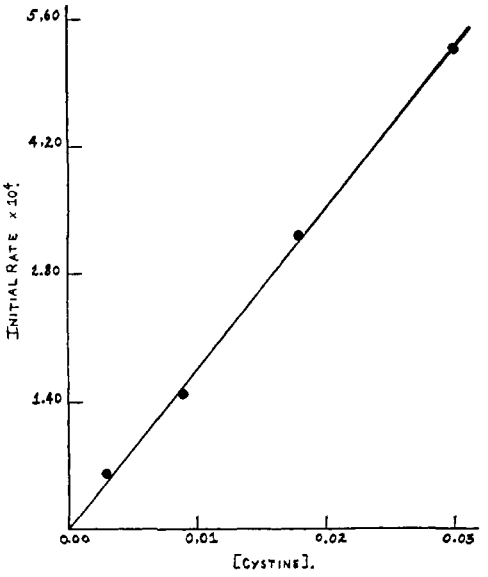

Fig. 1.—Effect of initial cystine concentration (molarity) on initial rate (moles × liter^{-1} × min.$^{-1}$) at 35° in 0.04 *M* potassium hydroxide.

removed under nitrogen. Under the conditions of the reaction, reactants and products were stable for the time periods employed in following the reaction.

Results and Discussion

The results are consistent with formulation of the reaction as

$$A + B \underset{2}{\overset{1}{\rightleftharpoons}} C + D \qquad (1)$$

$$D \xrightarrow{3} E \qquad (2)$$

where A, B, C, D and E represent, respectively, cystine, cyanide ion, cysteine, β-thiocyanatoalanine and 2-aminothiazoline-4-carboxylic acid.

Steady state treatment yields the kinetic equation

$$\frac{d(C)}{dt} = \frac{k_1 k_3 (A_0 - C)(B_0 - C)}{k_3 + k_2 C} \qquad (3)$$

For the early stages of the reaction, $k_3 >> k_2 C$, hence eq. 3 reduces to the bimolecular rate expression 4. Equation 3 also reduces to the bimolecular rate expression 4 if $k_2 << k_3$.

$$d(C)/dt = k_1(A_0 - C)(B_0 - C) \qquad (4)$$

In agreement with the above formulation, initial rates are linearly dependent on the cystine (Fig. 1) and cyanide (Fig. 2) concentrations, and kinetic data plotted (Fig. 3) according to the integrated form of the bimolecular rate expression give a straight line for 75% of the reaction course.[20] At 35° and in 0.040 *M* potassium hydroxide, the initial rate data yield a value for k_1 of 0.60 (±0.03) liter × mole^{-1} × min.$^{-1}$. A value of 0.62 liter × mole^{-1} × min.$^{-1}$ is obtained from the data of Fig. 3.

On integration, eq. 3 yields, for $[A_0] = [B_0]$

$$\frac{1}{(A_0 - C)} + \frac{k_2(A_0)}{k_3(A_0 - C)} + \frac{2.3 k_2 \log (A_0 - C)}{k_3} + \text{constant} = k_1 t \qquad (5)$$

(20) After 120 min., under the reaction conditions employed, destruction of cysteine becomes noticeable.

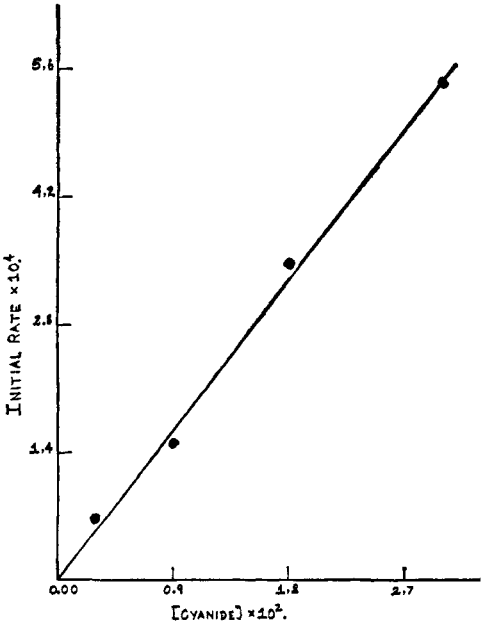

Fig. 2.—Effect of initial cyanide ion concentration (molarity) on initial rate (moles × liter^{-1} × min.$^{-1}$) at 35° in 0.04 *M* potassium hydroxide.

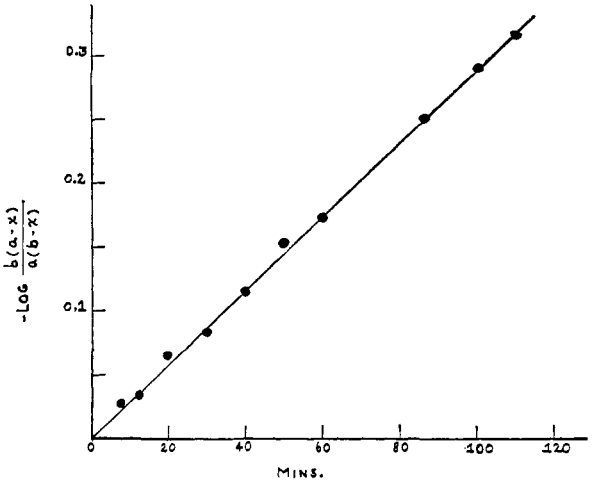

Fig. 3.—Plot of integrated bimolecular rate equation for kinetic run at 35°, 0.04 *M* potassium hydroxide; initial concentrations: cystine, 0.01 *M*; cyanide, 0.02 *M*.

A least squares calculation of the k_2 to k_3 ratio of eq. 5 from the data of a run at 35° for $[A]_0 = [B]_0 = 0.040$ *M* yielded the value 0.0083.

The bimolecular rate constant, k_1, calculated from eq. 5 for the above run had the value 0.56 (±0.06) liter × mole^{-1} × min.$^{-1}$ and from the integrated form of eq. 4 the value of 0.55 (± 0.06) liter × mole^{-1} × min.$^{-1}$ was obtained. It should be pointed out that the data fit both equations equally well and thus the ratio k_2/k_3, 0.0083, is not uniquely determined by the data. It is, of course, to be expected that $k_3 >> k_2$ and hence that thiocyanate ion could not be found in reaction mixtures[21,22]

(21) Thiocyanate ion was tested for with ferric iron and hydrochloric acid using the reaction conditions suggested by I. M. Kolthoff and E. B. Sandell, ref. 11, p. 456.

(22) When N,N¹-diacyl-cystines were treated with cyanide under the same reaction conditions, thiocyanate ion was found.

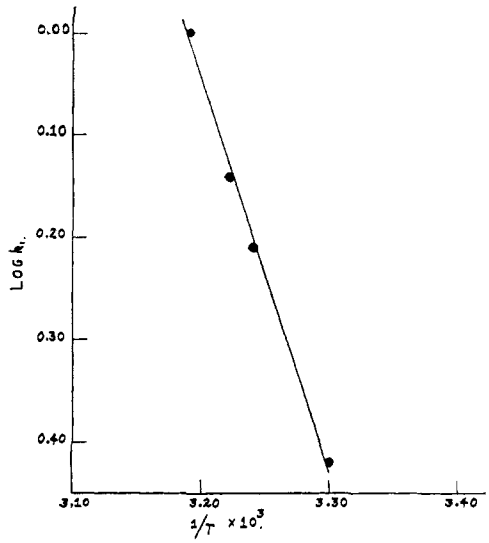

Fig. 4.—Temperature dependence of bimolecular rate constant, k_1; rate constants calculated from runs initially containing 0.01 *M* cystine and 0.02 *M* cyanide.

indicating that the cyclization reaction is also considerably faster than the displacement of the thiocyanato group with mercaptide ion, *i.e.*

$$RSCN + RS^- \longrightarrow R{-}S{-}R + SCN^-$$

The above bimolecular rate constant for the cystine–cyanide reaction at *p*H 12.4[23] is, of course, that for the reaction of cyanide ion with the di-anion of cystine and is of the same order of magnitude as that for the reaction of sulfite ion with the di-anion of cystine, 3 liters × mole^{-1} × min.$^{-1}$ at 25°.[24] It is also of interest to note that the effect of *p*H on the reaction rate is, as expected, complex: preliminary investigation at 40° yielded the bimolecular rate constants: 1.01 (*p*H 12.4), 0.79 (*p*H 11.5), 0.79 (*p*H 10.3) and at 99° and *p*H 4.75, 2.76.[25,26] The rate constant at 99° and *p*H 4.75 indicates the rate of reaction between hydrocyanic acid and cystine zwitterion to be quite slow compared to the reaction between cyanide ion and cystine di-anion. This may be attributed to the lower nucleophilicity of hydrocyanic acid as compared to cyanide ion, since in the sulfite reaction reaction rates with sulfite ion increase with positive charge on cystine ion, but decrease to negligible values when bisulfite ion is the reacting species.[24] Bimolecular rate constants in 0.040 *M* potassium hydroxide also were determined at several different temperatures, and a plot of log k *vs.* $1/T$ is presented in Fig. 4. The activation parameters at 35° are: E_a 16.8 kcal./mole, ΔH^* 16.1 kcal./mole and ΔS^* −7.4 entropy units. The entropy of activation is larger by a few units than the corresponding value for the reaction of cyanide ion (in methanol) with elemental sulfur,[2] but considerably smaller, by 20 e.u., than the value for the reaction of sulfite ion with cystine di-anion.[24] The latter difference is in agreement that cyanide ion is a stronger thiophile than the sulfite ion[2] and presumably is due to the fact that the sulfite–cystine activated complex is sterically more crowded than the cyanide–cystine activated complex.

The kinetics of the reaction between cyanide ion and N,N^1-diformyl-cystine[27] were also investigated, the reaction being followed by amperometric titration of thiol.[13,28] At 35° and *p*H 12.4, a bimolecular rate constant of 1.5 ± 0.3 liter × mole^{-1} × min.$^{-1}$ was found. The faster rate of reaction with the di-formyl compound may be attributed to the electron-withdrawing effect of the acyl groups, which may make the disulfide bond more susceptible to nucleophilic attack. The rate of reaction of N,N^1-diformyl-cystine with sulfite ion is similarly faster, by a factor of three, than the rate of reaction of cystine with sulfite.[24] In calculating the reaction rate constant, only initial data were used since thiocyanate ion appears subsequently in the reaction and because the reaction does not go to completion No thiazoline was detected in the reaction mixture, presumably because cyclization of β-thiocyanato-N-formyl-alanine to the thiazoline is prevented by the low nucleophilicity of the acylated nitrogen atom.[29]

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(23) The *p*H of the reaction mixture was measured with the glass electrode.

(24) R. Cecil and J. R. McPhee, "Advances in Protein Chemistry," Vol. XIV, Academic Press, Inc., New York, N. Y., 1959, p. 303.

(25) The reaction was conducted in sealed tubes; at 40° the reaction is very slow.

(26) H. Fraenkel-Conrat, *J. Am. Chem. Soc.*, **63**, 2533 (1941), has demonstrated the stoichiometry under these conditions.

(27) J. S. Fruton and H. T. Clarke, *J. Biol. Chem.*, **107**, 667 (1934).

(28) An aliquot of the reaction mixture was adjusted to *p*H 5.6 with acetate buffer and, after hydrocyanic acid was removed with nitrogen, an amperometric titration was carried out.

(29) The reaction of cyanide with di-acylated cystines is under further study in our laboratory.