The Reaction between Cyanide and the Mixed Disulphide of Cysteine and Penicillamine

By T. R. C. Boyde, Department of Clinical Biochemistry, University of Newcastle upon Tyne

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The Reaction between Cyanide and the Mixed Disulphide of Cysteine and Penicillamine

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Cyanide reacts with β3-dimethylcystine, but much more slowly than with cystine. The products are penicillamine and 2-amino-2-thiazoline-4-carboxylic acid.

The urine of individuals under treatment with penicillamine contains the mixed disulphide with cysteine (PeS·SCy);\(^1\) Lotz, Potts, and Bartter\(^2\) reported that this compound does not give a positive cyanide-nitroprusside test, but we have shown that a reaction does take place with cyanide, to yield penicillamine and 2-amino-2-thiazoline-4-carboxylic acid. No reaction could be detected between cysteine and penicillamine disulphide.\(^3\)

Cyanide reacts with cystine and related compounds by nucleophilic attack on sulphur, to yield a thiol and a thiocyanate. The thiocyanate reacts further to yield a more stable cyclic isomer.\(^4,5\) In unsymmetrical disulphides, there are three factors which may be considered to influence the direction of the reaction: \(^6\) the relative stabilities of the anions of the possible thiol products, the relative stabilities of the possible thiazoline (or thiazine) products, and steric hindrance. Probably the last is crucial in the case of PeS·SCy. One cannot compare the possible thiazoline products, since 2-amino-5,5-dimethyl-2-thiazoline-4-carboxylic acid has not been prepared. Although the alkyl substituents in penicillamine might be supposed to reduce the thiolate anion stability relative to the anion from cysteine, the thiol dissociation constants are, in fact, approximately equal (pK \(7.9\)).\(^7\)

The change in u.v. absorbance on treatment with cyanide forms a useful assay for either cystine or PeS·SCy. The conditions described below are especially convenient. Related disulphides, e.g. homocystine and the homocysteine-cysteine mixed disulphide, can also be estimated in this way. However, a sample solution with a low background absorbance is necessary, and this may demand laborious pre-treatment of samples from biological sources; there are complications if a thiol or another disulphide is also present.

Evidence is given below that cysteine catalyses the reaction between PeS·SCy and cyanide, probably through the exchange reaction:

\[
\text{PeS·SCy} + \text{CyS}^- \rightleftharpoons \text{PeS}^- + \text{CyS·SCy}
\]

Presumably this explains why cystine accelerates the reaction of PeS·SCy with cyanide, sufficient free cysteine being formed by reaction of the cystine with cyanide. Interference by cystine has made it difficult to apply the present results directly in a differential assay for PeS·SCy and cystine.

\(^4\) A. Schöberl, M. Kawohl, and R. Hamm, Chem. Ber., 1951, 84, 571.
EXPERIMENTAL

L-2-amino-2-thiazoline-4-carboxylic acid was prepared according to method 2 of Schöberl and Hamm. The material was finally recrystallized from 70% ethanol, which is known to yield the free acid; m.p. 235° (decomp.) (lit., 234°).


To a solution (3·0 mL) containing ββ-dimethylcystine (0·110 mg) and 0·050M-sodium tetraborate was added 10% aqueous potassium cyanide (0·1 mL). The solution was rapidly mixed and its absorbance recorded between 260 and 216 μm (Unicam SP 800 spectrophotometer, fast scan) within 1 min. of adding the cyanide and again 24 hr. later. The spectra are shown in the Figure, with the spectrum predicted from the presumed course of the reaction. The blank contained sodium tetraborate and cyanide, as above, and was treated in parallel with the test solution.

Change in u.v. absorbance when PeS·SCy is treated with cyanide: (····) PeS·SCy; (-----) 2-amino-2-thiazoline-4-carboxylic acid; (----) penicillamine (Sigma Chemical Co.); (-----) predicted absorbance; (-----) observed absorbance. All absorbances were determined for solutions in 48·5mm-sodium tetraborate containing 49·6mm-potassium cyanide, pH 9·3. The absorbance due to cyanide is negligible, so that the new absorbance at each wavelength can be calculated from ε(thiazoline) + ε(2-amino-2-thiazoline-4-carboxylic acid) - ε(PeS·SCy) - ε(CN⁻ - Na⁺), where ε is the molar extinction coefficient.

Kinetic Experiments.—Sodium tetraborate (0·075M; 2·0 mL) was pipetted into a spectrophotometer cuvette, and to this was added the sample solution and water to bring the volume to 3·0 mL. At zero time, 10% potassium cyanide (0·1 mL) was added and the solution was stirred. Readings of absorbance at 226 μm were taken at intervals of 1 min. for at least 5 min. and then at greater intervals for as long as the experiment demanded. The absorbance at zero time was determined by extrapolation. The pH after addition of

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cyanide was 9·3. Gawron et al. found that the optimum pH for the cystine-cyanide reaction was 8·9.

For samples containing cystine or PeS·SCy, the absorbance increased progressively to a maximum; this was reached in about 90 min. for cystine and about 24 hr. for PeS·SCy (25°C). After the maximum was reached, the absorbance of the PeS·SCy was stable, whereas that of the cystine samples decreased at about 3% per hr., presumably because of instability of the free cysteine produced.

The maximum absorbance increment in each case agreed well with that calculated from the molar absorbances of reactants and products (Figure). Reaction rates were therefore calculated directly from the observed rate of increase of absorbance and the appropriate molar absorbances.

Second-order rate constants were calculated for the reaction rates of cyanide with cystine and PeS·SCy, from the observed initial reaction rates. No correction was made for impurities in any of the reagents. The results were:

- cystine; \( k' = 1·6 \text{ l. mole}^{-1} \text{ min}^{-1} \)
- PeS·SCy; \( k' = 0·1 \text{ l. mole}^{-1} \text{ min}^{-1} \)

A sample containing penicillamine disulphide (123 μg.) was treated as described above. There was no detectable change of absorbance during 140 min.

If mixtures of cystine and PeS·SCy were used, the reaction rate was greater than would have been expected from the rates obtained with each alone at the same concentrations. A corresponding effect was obtained with cystine. If cysteine was added to the PeS·SCy samples the rate of increase of absorbance was enhanced (cysteine alone showed no change of absorbance, as expected). The rate was approximately doubled in the presence of 0·065mM-cysteine and trebled in 0·13mM-cysteine. The concentration of PeS·SCy used was 0·07mM.

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