BBA 26168

THE PHOTOCHEMICAL DEGRADATION OF CYSTINE IN AQUEOUS SOLUTION IN THE PRESENCE OF AIR

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(Received March 6th, 1969)

SUMMARY

1. Ultraviolet irradiation of cystine in air-saturated aqueous solution at pH 1 and 10 has been investigated.

2. The quantum yield for destruction of cystine has been shown to be independent of pH, the increase in destruction rate at pH 10 being explained by the increased absorption of energy at this pH. The reaction shows first-order characteristics when the intensity of the incident is in excess of that absorbed by cystine.

3. Degradation of cystine occurs only when the wavelength of the incident radiation is below 300 nm.

4. A large number of products have been confirmed, notably pyruvic acid, cysteine, ammonia, cysteic acid, alanine 3-sulphinic acid, cysteine *S*-sulphonic acid, alanine, serine, glycine, SO_4^{2-} and, in acid solution, lanthionine, bis-(2-amino-2-carboxyethyl)trisulphide, bis-(2-amino-2-carboxyethyl)tetrasulphide and H_2S. NH_2OH has been identified at both pH values.

5. It has been deduced that the difference in products formed in acid and alkali is due to secondary reactions rather than to any difference in the original fission of the cystine links.

6. Evidence suggests that fission of C–S, S–S and C–N links occur in acid and alkaline solution.

7. No decarboxylation products of cystine have been detected.

8. A reaction mechanism has been postulated which accounts for the products identified and for the amounts formed.

INTRODUCTION

Ultraviolet irradiation of proteins[1] causes degradation of several amino acids including cystine[2,3]. It has been suggested that the inactivation of enzymes[4,5] and the yellowing of keratin fibers[6–9] may be due to this decomposition. The earlier work on cystine degradation has been well reviewed by SAVIGE AND MACLAREN[10]. In some of this work experimental detail is lacking, and the products formed were difficult to identify without modern chromatographic techniques. MORI[11], using paper chromatography, was able to identify many of the simpler products from the irradiation of cystine solutions by a mercury vapor lamp. In all of this earlier work no products containing the intact S–S bond were reported.

SAVIGE and coworkers[12–15] have studied the photochemical degradation of cystine in greater detail. By using several ultraviolet sources, they obtained data which they tried to correlate with such parameters as pH of solution, concentration of cystine and the predominent wavelength of incident radiation. Many products were reported, some of which were only formed in trace amounts and some of which contained the intact S–S bond. Recently pyruvic acid formation has been suggested as arising *via* α-amino-α′-oxo-β,β′-dithiodipropionic acid during irradiation of cystine solutions[16].

The present work presents quantitative data on the production of the major products of which many have been previously qualitatively reported.

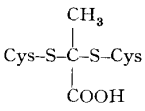
EXPERIMENTAL

Materials

The following commercially available chemicals used were L-cystine, pyruvic acid (Hopkin and Williams), L-cysteine·HCl, L-cysteic acid, DL-alanine, DL-serine, glycine, taurine, NH_2OH (B.D.H.), L-thiazolidene-4-carboxylic acid (K and K Labs.) and DL-lanthionine (Nutritional Biochemicals Corp.). Where necessary the amino acids were recrystallized. DL-Diaminoadipic acid was prepared by the method of SHEEHAN AND BOLHOFFER[17]; alanine 3-sulphinic acid by the method of EMILIOZZI AND PICHAT[18]; cysteine *S*-sulphonic acid by the method of SÖRBO[19]; and bis-(2-amino-2-carboxyethyl)trisulphide by the method of FLETCHER AND ROBSON[20]. Bis-(2-amino-2-carboxyethyl)tetrasulphide was prepared in solution by allowing the trisulphide to stand in dilute HCl. Automatic amino acid analysis of this solution showed two extra peaks of which one belonged to cystine and the other presumably was due to the tetrasulphide formed according to the equation[20]:

$$2\ \text{Cys–S–S–S–Cys} \longrightarrow \text{Cys–S–S–Cys} + \text{Cys–S–S–S–S–Cys}.$$

The impure adduct of pyruvic acid and cysteine was prepared by the method of SCHUBERT[21]. On heating in aqueous solution at 100°, this compound gives impure thiazolidene-2-methyl-2,4-dicarboxylic acid[22]. The presence of the latter was confirmed by the synthesis of RIVETT *et al.*[23]. While the structure of the former compound is unknown, the simplicity of its formation suggests that it may be the mercaptal



Ultraviolet spectra of the cystine solutions

The absorption spectra were determined on a Hilger Watts Uvispec H700. 0.40 g cystine was dissolved in 1 l of distilled water containing either 10 ml of concentrated HCl or 10 ml of 0.880 M ammonia. The absorbance of these solutions was plotted against wavelength with the solvent as blank.

Preparation of the cystine solutions

Acid solutions of cystine were prepared by dissolving 1.0 g cystine in 100 ml distilled water containing 10 ml A.R. 35 % HCl and by diluting it to 1 l with distilled

water. Alkaline solutions were made by dissolving 1.0 g cystine in 500 ml distilled water containing 10 ml A.R. concentrated ammonia solution and by diluting it to 1 l with distilled water. All solutions contained 4.15 μmoles cystine/ml.

Irradiation of cystine solutions

All the solutions were irradiated with a Hanau type Q81 high pressure mercury vapor immersion lamp protected by a quartz sheath. The solutions were circulated as a 7-mm layer around the lamp at a constant rate with a peristaltic pump. The temperature was maintained at 15°. Samples were withdrawn for analysis as required from the open reservoir included in the cycle. 1 l of solution was used for each irradiation and all samples for amino acid analysis were immediately freeze-dried on withdrawal in order to reduce the possibility of subsequent reaction.

Analysis of irradiated solutions

High voltage electrophoresis was carried out on 15-cm wide Whatman 3MM paper for 120 min between 1 m plates with a potential gradient of 100 V/cm at 5°. The buffer (pH 1.85) consisted of 25 g formic acid and 78 g acetic acid diluted with distilled water to 1 l. The residue from a 2-ml freeze-dried sample was diluted to 0.2 ml with distilled water; 50-μl aliquots were applied to the paper. After electrophoresis was carried out, the paper was dried at 80° and was developed with cadmium acetate–ninhydrin reagent[24]. The major bands were identified by direct comparison with controls of known materials. Subsequent chromatography in the second dimension with *n*-butanol–glacial acetic acid–water (4:1:1, by vol.) showed that electrophoresis had resolved the bands into single substances.

Quantitative amino acid analyses were carried out on a Technicon autoanalyser using the single column method of HAMILTON[25] with norleucine as an internal standard.

Pyruvic acid was estimated colorimetrically by the method of FRIEDMANN and HAUGEN[26]. Samples were analysed immediately without being freeze-dried.

Ammonia was estimated colorimetrically with Nessler's reagent[27]. Analysis was carried out immediately without freeze-drying.

Cysteic acid was estimated by electrophoresis at pH 1.85 (ref. 28).

To detect NH_2OH after a 10-h irradiation, a 100-ml sample was taken to dryness in a rotary evaporator at 20°. NH_2OH was identified in this sample as the hydroxamic acid[29]. This test was not sensitive enough to follow its formation quantitatively.

To test for peroxides to 10 ml of the acidified irradiated solutions 3 ml amyl-alcohol were added, and the mixture was shaken with three drops of $K_2Cr_2O_7$ solution. In the presence of peroxide a blue color should develop in the organic layer. No color formed in the irradiated samples, while controls to which traces of H_2O_2 were added developed immediate color.

RESULTS AND DISCUSSION

On exposure of cystine to ultraviolet radiation from the immersed high pressure lamp, destruction of cystine commenced immediately and was virtually complete after a 10-h exposure. A more intense yellow color developed when cystine was exposed at pH 10. This difference has been previously noted[30], but the yellow pigment

has not yet been identified. Despite this observed difference in the rate of yellowing, the rate of decomposition of cystine on exposure to ultraviolet radiation is not so dependent on pH. The initial decomposition of cystine shows first-order kinetics with respect to the cystine concentration under the experimental conditions used, *i.e.*, intensity of radiation in excess of that absorbed by the cystine, and a plot of log concentration of cystine against time is linear at both pH values. The color is therefore probably developed through secondary pH-dependent reactions. The rate is slightly greater at pH 10 ($K = 6.54 \cdot 10^{-3}$) than at pH 1 ($K = 5.50 \cdot 10^{-3}$). The quanta actually absorbed by the cystine during the irradiations have been calculated for the relevant line outputs of the lamp (Tables I and II); from these values the total number of

TABLE I

CALCULATED QUANTA OF ENERGY ABSORBED BY THE IRRADIATED ACIDIC CYSTINE SOLUTIONS AT VARIOUS WAVELENGTHS OF LIGHT (INITIAL VALUE)

Wavelength of line (nm)	*Quanta/sec from lamp** ($\times 10^{18}$)	*Absorbance of solution***	*Transmission of solution* (%)	*Absorption of solution* (%)	*Quanta/sec absorbed by solution* ($\times 10^{18}$)
248	0.324	0.900	13.0	87.0	0.282
254	1.59	0.825	15.0	85.0	1.35
265	0.688	0.577	26.5	73.3	0.507
270	0.074	0.463	34.5	65.5	0.049
280	0.305	0.263	54.7	45.3	0.138
289	0.276	0.140	72.5	27.5	0.076
297	0.693	0.070	85.0	15.0	0.103
302	1.24	0.040	91.0	9.0	0.111
313	2.64	0.017	96.0	4.0	0.105
					Total 2.721

* Supplied by the manufacturers.
** Corrected for path length and concentration.

TABLE II

CALCULATED QUANTA OF ENERGY ABSORBED BY IRRADIATED ALKALINE CYSTINE SOLUTIONS AT VARIOUS WAVELENGTHS OF LIGHT (INITIAL VALUE)

Wavelength of line (nm)	*Quanta/sec from lamp** ($\times 10^{18}$)	*Absorbance of solution***	*Transmission of solution* (%)	*Absorption of solution* (%)	*Quanta/sec absorbed by solution* ($\times 10^{18}$)
248	0.324	1.03	9.3	90.7	0.293
254	1.59	1.00	10.0	90.0	1.43
265	0.688	0.850	15.7	84.3	0.582
270	0.074	0.675	21.3	78.7	0.058
280	0.305	0.428	37.0	63.0	0.190
289	0.276	0.228	59.0	41.0	0.113
297	0.693	0.131	74.0	26.0	0.180
302	1.24	0.086	82.0	18.0	0.222
313	2.64	0.025	95.0	5.0	0.132
					Total 3.200

* Supplied by the manufacturers.
** Corrected for path length and concentration.

TABLE III

THE INITIAL QUANTUM EFFICIENCY OF THE DECOMPOSITION OF CYSTINE IN ACIDIC AND ALKALINE SOLUTION

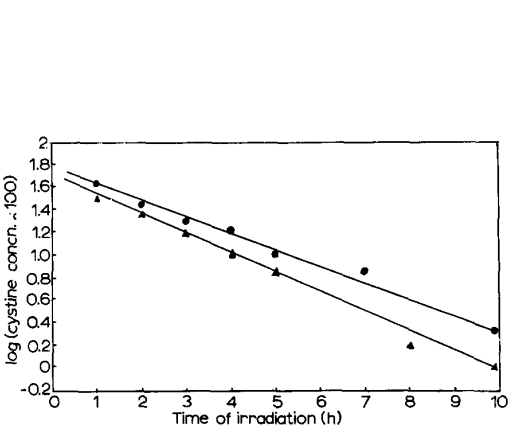
Solutions initially contained 4.15 μmoles/ml.

pH of solution	*Percentage of total cystine decomposed*	*Number of moles decomposed*	*Number of molecules decomposed/sec**	*Φ***
1	58	0.00242	$4.03 \cdot 10^{17}$	0.15
10	68	0.00283	$4.73 \cdot 10^{17}$	0.15

* An average value over the first hour.

** Φ is quantum efficiency, *i.e.*, number of molecules decomposed/sec per number of quanta absorbed/sec.

quanta initially absorbed per sec by the cystine is found to be $2.72 \cdot 10^{18}$ in acid and $3.20 \cdot 10^{18}$ in alkali. This 18% increase in absorption at the higher pH explains the 19% increase in the decomposition rate of cystine at this pH. From the concentration curves (Fig. 1), the number of molecules destroyed per sec (average value over the 1st hour) was found to be $4.03 \cdot 10^{17}$ in acid and $4.73 \cdot 10^{17}$ in alkali (Table III). The quantum efficiency of the initial reaction was found to be independent on pH and to have a value of 0.15 which is of the same order as the previously quoted value of 0.13 (ref. 32) found in acid solution under N_2. This suggests that the rate of the initial fission is independent of the O_2 concentration. This behavior is consistent with a free radical mechanism for the initial breakdown of the cystine molecule. Previous authors using different techniques, *e.g.* polymerization initiation[31], electron spin resonance measurements[14], disulphide interchange[15], *etc.*, on irradiation of disulphide



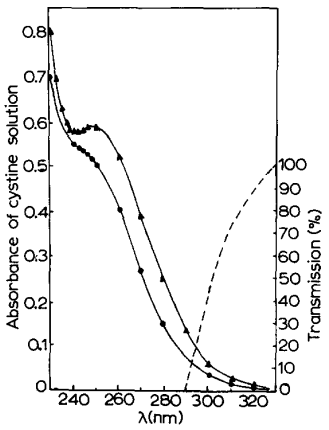


Fig. 1. The rate of decomposition of cystine plotted as log cystine concn. against time. Concn. expressed as μmoles of cystine remaining per μmole of cystine originally present. ●—●, at pH 1; ▲—▲, at pH 10.

Fig. 2. The ultraviolet absorption spectra of cystine solutions showing the influence of pH on the absorption maximum. ●—●, at pH 1; ▲—▲, at pH 10; – – – –, transmission of the pyrex glass filter.

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