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OXIDATION OF CYSTEINE WITH CHLORAMINE-T AND DICHLORAMINE-T

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THE oxidation of cysteine and other thiols[1] has been the subject of a number of investigations. The sulphydryl group is generally oxidized to the corresponding disulphide, although instances of oxidation beyond the disulphide stage are known[2,3]. Kinetic investigations of the oxidation of thiols as a rule require rapid analytical techniques. Cysteine is an important amino acid and several analytical reagents[4-11] have been employed for assaying this compound by direct or through instrumental methods. In the present investigations, we have examined the behaviour of chloramine-T (CAT) and dichloramine-T (DCT) towards cysteine and we are reporting about some simple analytical methods devised for estimating the compound, with these reagents.

Materials.—L(+)-Cysteine (E. Merck) was purified by recrystallization from aqueous solution and was assayed to 95·5% by the iodimetric method[4]. The purity of the sample was checked by TLC and paper chromatographic methods, where it gave a single spot. An aqueous solution (~ 2 mg per ml) was prepared by dissolving the compound in water containing a few drops of dilute HCl. Other solutions of cysteine were prepared by dissolving the solid in appropriate buffers[12] and solvents.

CAT (E. Merck) was purified by the method of Morris *et al.*[13]. An approximately decinormal solution was prepared and standardized by the iodometric method. DCT was prepared and standardized by the method of Jacob and Nair[14]. Reagent grade materials were used in preparing solutions of other compounds. All solutions were prepared in triple distilled water.

Two methods were adopted for estimating the thiol.

(I) *Direct Titration Procedure* : Preliminary experiments showed that aqueous cysteine solutions can be directly titrated against CAT with starch-KI internal indicator in presence of dilute H_2SO_4. The overall concentration of H_2SO_4 should be maintained at least at 0·2 N and higher acid concentrations did not affect the results. In the recommended procedure, aliquots of aqueous cysteine solution are taken in a titration flask. About 2 ml of starch-KI mixture and enough of 2 N H_2SO_4 to make the overall concentration 0·2 N are added. The solution is titrated against standard CAT solution to the appearance of a pale blue colour. The values are found to be reproducible.

A potentiometric titration between aqueous cysteine and CAT solutions was found to be unsuitable as no sharp potential break could be observed under the experimental conditions.

A direct titration of cysteine in glacial acetic acid solution against a 0·02192 N solution of DCT (in glacial acetic acid) was carried out potentiometrically and by visual end-point method as described for thioglycolic acid[15]. A potential break of about 100 mv was recorded for a 0·1 ml addition of titrant near the end-point.

Oxidation of the thiol involves a single electron change with both CAT and DCT, which can be stoichiometrically represented as :

$$2\ R'SH + RN\ Cl\ Na \rightarrow R'SSR' + RNH_2 + NaCl \quad (1)$$

$$4\ R'SH + RNCl_2 \rightarrow 2\ R'SSR' + RNH_2 + 2\ HCl \quad (2)$$

where $R' = HOOC.CH(NH_2)\ CH_2$ and $R = p\text{-}CH_3 - C_6H_4SO_2$. The presence of disulphide (R_f = 0·044) was detected by paper chromatography with butanol-acetic acid-water (4 : 1 : 5 v/v) as solvent and ninhydrin spray reagent (0·2% solution in butanol-water-acetic acid 95 : 4 : 0·5 v/v).

Some typical results of analyses are given in Table I. It can be seen that CAT and DCT can be used for a rapid and accurate assay of thiol.

The interference of some amino acids and related compounds in the estimation of cysteine was investigated. Lysine, leucine, glutamine, methionine and urea (~ 0·1 m mole) did not interfere in the estimation with CAT, while histidine, alanine, valine, serine, threonine, arginine, glycine, proline and thiourea interfered. Although a similar behaviour was noticed with DCT titrations, leucine and glutamine interfered while arginine, glycine, proline and urea had no effect.

(II) *Back Titration Procedure* : In preliminary experiments with CAT, known amounts of cysteine solution (~ 10 mg) prepared in the appropriate buffer were added to a known excess volume of CAT solution (~ 1·25 m moles) in an iodine flask. The reaction mixture was set aside for various intervals of time, with occasional shaking. Then the excess CAT left unconsumed was determined

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TABLE I

Oxidation of cysteine with chloramine-T and dichloramine-T

Titrant CAT		Titrant DCT				Titration CAT	
Direct titration		Direct titration		Potentiometric titration		Back titration method	
Weight of thiol		Weight of thiol		Weight of thiol		Weight of thiol	
Taken mg	Found mg	Taken mg	Found mg	Taken mg	Found mg	Taken mg	Found mg
9·26	9·26	9·92	9·96	10·04	10·09	5·96	6·01
18·52	18·51	19·83	20·18	20·07	20·19	9·93	9·92
27·78	27·62	34·70	34·80	30·11	29·89	19·86	19·91
37·04	36 88	44·61	44·61	40·15	40·12	23·84	23·69
57·23	57·51	49·56	49·66	50·19	50·73	29·80	29·55
76·31	76·31	59·48	59·60	60·22	60·28	33·77	33·67
95 39	95·96	69·39	69·46	..	..	36·60	36·61

by back titration. A comparison of the extent of oxidation after 30 min. showed that there is a 10 electron change per mole of cysteine in buffer media of pH 1–3. which decreases with the increase in pH. *i.e.*, 9·5 at pH 5, 8·8 at pH 7 and 6·3 in 0·1 N NaOH. The results were quite reproducible in the pH range 1–3 and the following procedure was therefore used for estimating the thiol. A solution of cysteine in pH 1 buffer (~ 2 mg/ml) was prepared. Aliquots of the solution were added to 50 ml of decinormal CAT solution in an iodine flask. The mixture was shaken occasionally and after 30 min. 10 ml of 2 N H_2SO_4 and 10 ml of 20% KI were added and the liberated iodine was titrated against standard thiosulphate. The amount (x mg) of cysteine in the experimental solution is given by

$$x = 12{\cdot}12\, y\,(v_1 - v_2),$$

where y is the normality, v_1 is the blank titration and v_2 the volume of thiosulphate used to titrate the excess of CAT after oxidation of cysteine.

Stoichiometry of the above oxidation could probably be represented as follows :

$$HOOC - CH(NH_2)\,CH_2SH + 5\,RNCl\,Na + 5\,H_2O \rightarrow HOOC - CH(NH_2)\,CHO + 5\,RNH_2 + 5\,NaCl + H_2SO_4. \quad (3)$$

Paper chromatography was used to identify the reaction products. Benzyl alcohol saturated with water was used as the solvent for detecting the sulphonamide ($R_f = 0{\cdot}905$) and 0·5% vanillin in 1% HCl solution in ethanol was the spray reagent. Attempts were made to detect the aldehydic amino acid with the amino acid solvent and spray reagent, employed for the disulphide. A spot corresponding to $R_f = 0{\cdot}091$ was observed and this probably can be taken as evidence for the compound.

Typical results of analyses are shown in Table I. The back titration procedure with DCT was unsatisfactory as the reaction was sluggish and showed only a 5–6 electron change per mole of cysteine (in glacial acetic acid solution) even after 40 minutes.

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