

Action of Sodium Hypochlorite on α -Amino Acids

A. KANTOUCH and S. H. ABDEL-FATTAH

*Textile Laboratory, National Research Centre,
Dokki, Cairo (U.A.R.)*

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The action of sodium hypochlorite on some α -amino acids as glycine, serine, tyrosine, cystine, aspartic acid, lysine and arginine has been investigated. It was observed that chlorine reacted very quickly with most of these amino acids leading to the oxidation and/or formation of chloro derivatives of the amino acids, depending on the pH of the reaction. At pH 2 and 25°C, oxidation occurs mainly through the formation of the intermediates *N*-chloro or *N*-dichloro derivatives. Substitution of one of the hydrogen atoms of the amino groups by an acetyl group decreased considerably the rate of oxidation. A mechanism of the action of sodium hypochlorite on these amino acids is suggested.

Though some works dealt with the action of chlorine or hypochlorite on proteins, peptides and free amino acids, no adequate explanation for the reactions taking place between these substances and chlorine has been found in the literature. However, *Wright* [1, 2] reported that acidity of the reaction medium increased chlorination while alkalinity increased the oxidation of such substances by hypochlorite. *Alexander* and *Gough* [3] reported that most of the amino acids reacted rapidly with chlorine in acid and alkaline solutions and they noted that with the exception of glycine all amino acids were readily oxidized by chlorine at pH 2 and 10. Other authors [4, 5] stated that chloramines are formed and free active chlorine reacts with free amino acids giving rise to oxidation or chlorination products depending on the pH and temperature of the reaction. Reaction of the free chlorine with amino acids in strong acid solution catalyzed by the ultraviolet radiation has been reported [6] to give compounds mainly substituted on the methylene groups of the amino acid chains. Formation of chlorination products by a radical chlorination of α -amino acids using ultraviolet radiation was also reported [7].

In the present work the action of sodium hypochlorite on glycine, serine, tyrosine, cystine, aspartic acid, lysine and arginine has been studied.

Experimental

The amino acids used in this investigation were of A.R. grade.

Sodium hypochlorite used as a chlorinating agent was prepared by passing a stream of chlorine gas in A.R. sodium hydroxide.

Chlorination of amino acids investigated was carried out as follows: 10 mmoles of amino acid were treated with varying amounts of chlorine in 100 ml solution at pH 2 or pH 8. The reaction was allowed to proceed for different periods up to 120 minutes at 25°C.

N-Acetylglycine and *N,N'*-diacetyl-L(-)cystine have been prepared according to [8] and [9], respectively.

The total chlorine consumption was estimated iodometrically using 5 ml aliquots from the reaction solution. The titration was carried out immediately to avoid any reaction which might take place between the liberated iodine and any free amino acid. Free chlorine was estimated as follows: solution (5 ml) was shaken vigorously with 30 ml of cooled carbon tetrachloride (10°C). The carbon tetrachloride layer was separated and its chlorine content was titrated with 0.1 N sodium thiosulfate. Correction for any free chlorine in the aqueous layer was done using the partition coefficient of chlorine between water and carbon tetrachloride. Remaining active chlorine in the aqueous layer corresponding to *N*-chloro derivatives was also estimated iodometrically.

Chromatographic analysis of free amino acids was carried out as follows: Aliquots of reaction solution which had been freed from any free chlorine were developed by two-dimensional paper chromatography using acetic acid—butanol—water mixture in the first direction and phenol—water mixture in the second direction. In some experiments this separation was done using low voltage ionophoresis technique [10, 11]. The chromatogram was developed with 0.2% ninhydrin solution in acetone and the resulting spots were compared with reference amino acids. In the case of aldehydes the dinitrophenyl hydrazone of any formed aldehydes of the reaction mixture was also developed on separate chromatograms [12].

Results and Discussion

Chlorination of glycine

The course of the chlorine consumption during chlorination of glycine is shown in Fig. 1.

To study the state of available chlorine during chlorination of glycine, a new method was developed for the estimation both of free chlorine and *N*-chloro deri-

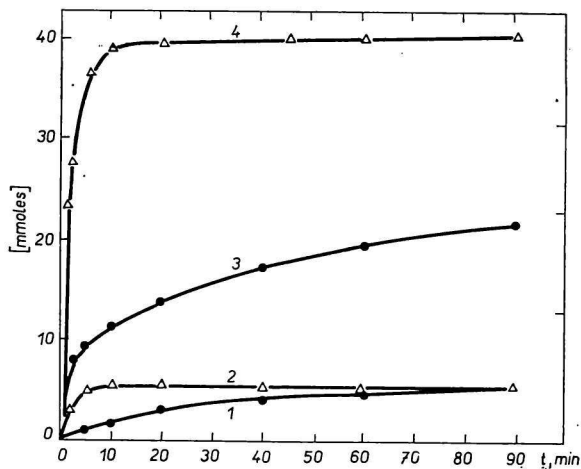


Fig. 1. The chlorine consumption (mmoles) during chlorination of glycine (10 mmoles) at 25°C.

● pH 2; △ pH 8.

1., 2. 10 mmoles of chlorine; 3., 4. 40 mmoles of chlorine.

vatives of amino acids. Free chlorine was extracted from aqueous solution with carbon tetrachloride while the amino acid *N*-chloro derivative was left in solution. The results indicate that glycine reacts with nearly equivalent amounts of chlorine to give rise to *N*-chloro and *N*-dichloro derivatives. Dependence of the free chlorine content on the reaction time is illustrated in Fig. 2. The rate of chlorine consumption and the rate of decrease in *N*-chloro derivatives are nearly the same and this may be taken as evidence that oxidation takes place through the formation of some intermediates. It might be assumed that the *N*-chloro derivatives decompose giving rise to an autoxidation of glycine and formation of some free chlorine.

At pH 8, the rate of chlorine consumption depends mainly on the initial chlorine content (Fig. 1). On using 10 mmoles of chlorine, the rate was found to be the same as that of pH 2. With 40 mmoles of chlorine, oxidation occurred very rapidly and chlorine disappeared completely from the reaction medium in few minutes. On the other hand more than one hour was necessary for the complete consumption when 20 mmoles of chlorine were used. The initial rate of chlorine consumption at pH 8 which is higher than that at pH 2, could be ascribed to higher resistance of *N*-chloro derivatives formed at pH 2 to oxidation. The high oxidation rate at pH 8 may also be due to the formation of intermediates sensitive to alkaline oxidation.

The results found for glycine oxidation contradict the results of *Alexander* and *Gough* [3] who reported that glycine was readily oxidized by chlorine at pH 2 and pH 10. Our results are in agreement with those of *Norman* [13] who reported that the reaction mixture became more acidic as oxidation proceeded.

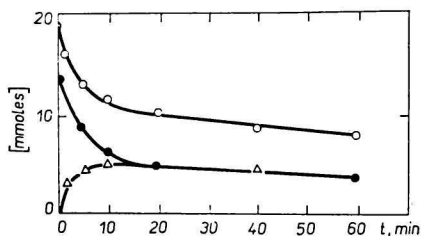


Fig. 2. The state and content of available chlorine (mmoles) during chlorination of 10 mmoles of glycine (20 mmoles of chlorine, pH 2, 25°C).

● chloramine; △ free chlorine; ○ total active chlorine.

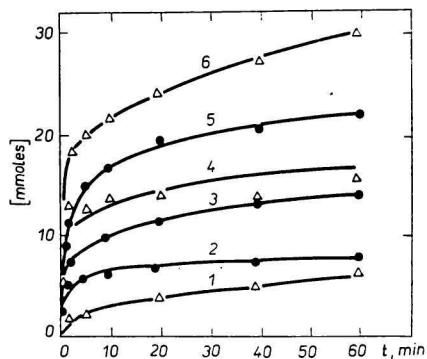


Fig. 3. The chlorine consumption (mmoles) during chlorination of serine (10 mmoles) at 25°C.

● pH 2; △ pH 8.
1., 2. 10 mmoles of chlorine; 3., 4. 20 mmoles of chlorine; 5., 6. 40 mmoles of chlorine.

The role of the free amino group in the reaction with chlorine has also been investigated. For this purpose, a chlorination of acetyl glycine was carried out. The results (Table 1) show that only the substitution of the free hydrogen atom of the amino group takes place. Complete substitution took place after one hour in the case of chlorine content (10 mmoles) and in about two minutes in the case of

Table 1

Chlorination of acetylglycine at pH 2 and 25°C

Reaction time [min]	Chlorine concentration in reaction medium (mmoles/100 ml)			Active chlorine consumed (mmoles)	Chlorine concentration in reaction medium (mmoles/100 ml)			Active chlorine consumed (mmoles)
	Chlor-amine	free	total		Chlor-amine	free	total	
0	—	—	10.60	—	—	—	21.30	—
0.3	3.07	7.50	10.57	—	7.50	14.15	21.65	—
1	5.62	5.03	10.65	—	9.20	11.60	20.80	0.50
2	7.10	3.26	10.36	—	10.70	9.90	20.60	0.70
5	8.55	2.20	10.75	—	11.40	8.70	20.10	1.20
10	9.05	1.70	10.75	—	11.40	8.00	19.40	1.90
20	9.60	0.96	10.56	—	11.40	7.60	19.00	2.30
40	9.85	0.72	10.57	—	11.40	7.00	18.40	2.90
60	10.00	0.54	10.54	—	11.40	6.80	18.20	3.10

chlorine content (20 mmoles). Oxidation of acetylglycine is negligible if compared with glycine; little oxidation is observed only with 20 mmoles of chlorine content. This may be attributed to the formation of traces of dichloro derivatives as a result of some deacetylation reaction and may imply that *N*-dichloro derivatives play the main role in the oxidation process of glycine.

Chlorination of serine

The action of chlorine on serine was investigated at pH 2 and at pH 8 using 10 mmoles of serine in a solution (100 ml) (Fig. 3). It is apparent that at pH 2 serine consumes different amounts of chlorine depending on the initial chlorine content. The rate of reaction is relatively high at the start of the treatment and then gradually decreases. No complete chlorine consumption was observed after one-hour treatment.

Chloramine formed by the effect of chlorine on this amino acid was found to be equivalent to about 60% of the available chlorine after 20 seconds of treatment, when using 10 mmoles of initial chlorine content. On the other hand, the chloramine formed in the case of 20 mmoles of chlorine indicated formation of *N*-monochloro serine while at 40 mmoles the *N*-dichloro derivative was formed. While chloramine content decreased progressively, free chlorine in the reaction solution increased gradually at the start of reaction and then slightly decreased (Fig. 4). This phenomenon may be caused by decomposition of the formed *N*-chloro and *N*-dichloro intermediates. The relatively rapid disappearance of *N*-chloro serine derivatives from reaction medium indicates higher reactivity of these compounds as compared with glycine derivatives (Fig. 2).

Chlorination of tyrosine

At pH 2 (Fig. 5), chlorine is very rapidly taken out from the solution of tyrosine with the formation of brown precipitate. A relatively small amount of available chlorine remains in solution in the form of free chlorine or chloramine. Tyrosine reacts rapidly with sodium hypochlorite and the reaction is complete in few minutes on using 10 and 20 mmoles of the chlorine content per 10 mmoles of amino acid.

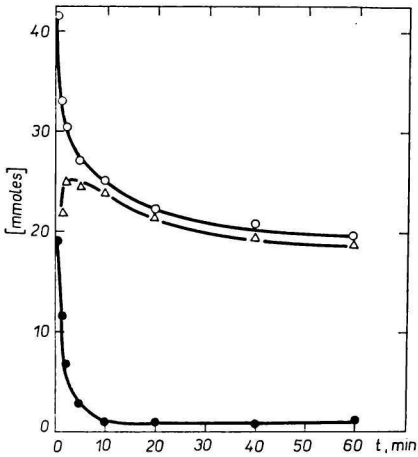


Fig. 4. The state and content of available chlorine (mmoles) during chlorination of 10 mmoles of serine (42 mmoles of chlorine, pH 2, 25°C).

● chloramine; Δ free chlorine; \circ total active chlorine.

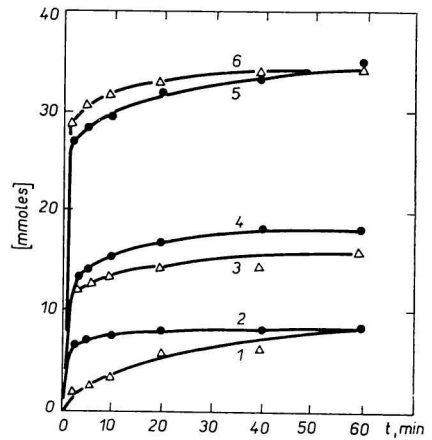


Fig. 5. The chlorine consumption (mmoles) during chlorination of tyrosine (10 mmoles) at 25°C.

● pH 2; Δ pH 8.

1., 2. 9.5 mmoles of chlorine; 3., 4. 19 mmoles of chlorine; 5., 6. 37 mmoles of chlorine.

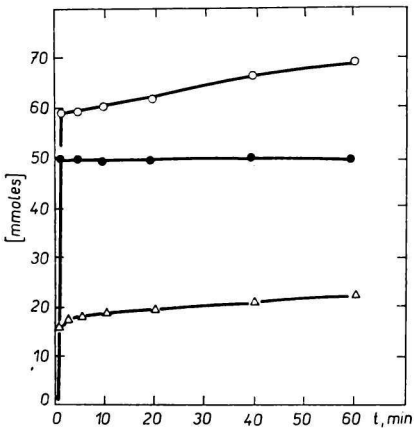


Fig. 6. The chlorine consumption (mmoles) during chlorination of cystine (10 mmoles) at 25°C.

● pH 2, 50 mmoles of chlorine; \circ pH 2, 70 mmoles of chlorine; Δ pH 8, 40 mmoles of chlorine.

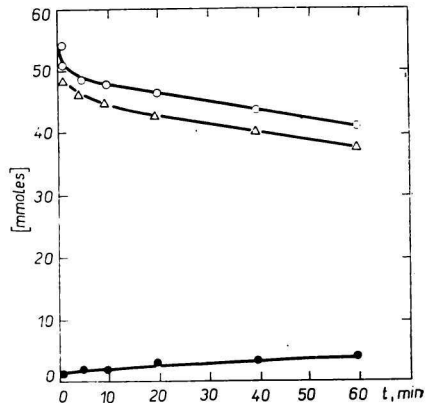


Fig. 7. The state and content of available chlorine (mmoles) during chlorination of 10 mmoles of N,N' -diacetyl cystine (50 mmoles of chlorine, pH 2, 25°C).

● chloramine; Δ free chlorine; \circ total active chlorine.

At pH 2, tyrosine forms 3-chloro and 3,5-dichloro derivatives which can be detected by two-dimensional paper chromatography. The reaction results indicate that at this pH two reactions take place. The first one is the chlorination reaction which gives rise to *N*-chloro tyrosine beside 3-chloro and 3,5-dichloro derivatives, while the second one is the oxidation and takes place as the result of decomposition of the formed *N*-chloro derivatives.

At pH 8, a rapid chlorine consumption occurs at the start of the treatment, depending on the amount of hypochlorite used. The chlorine consumption slows down as the reaction proceeds and is complete in about one hour. At this pH only oxidation reaction is observed probably leading to the formation of oxyphenyl-acetaldehyde [14].

Chlorination of cystine and cysteic acid

Cystine was found to remove chlorine immediately from its solution at pH 2 on using 10 and 50 mmoles of available chlorine (Fig. 6). The use of 70 mmoles of chlorine leads to the formation of cysteic acid in nearly quantitative yield beside a small amount of *N*-chloro derivatives. This may be due to the relatively low amount of chlorine and/or formation of strongly acidic sulfonic groups which, by zwitterion formation with the amino group, make this amino group less reactive to chlorination. It may also be due to the high instability of any formed chloramine which is consumed for the oxidation as soon as it is formed. We have found that the available chlorine in solution exists as a free chlorine beside a small amount of chloramine. When chloramine was completely consumed, oxidation took place at the expense of the present free chlorine which decreased gradually.

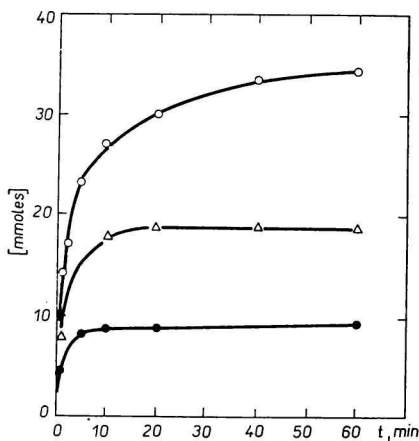


Fig. 8. The chlorine consumption (mmoles) during chlorination of aspartic acid (10 mmoles) at pH 2 and 25°C.

● 9.5 mmoles of chlorine; Δ 19 mmoles of chlorine; ○ 38 mmoles of chlorine.

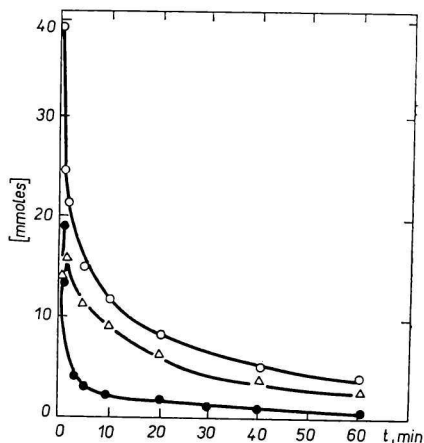


Fig. 9. The state and content of available chlorine (mmoles) during chlorination of 10 mmoles of aspartic acid (40 mmoles of chlorine, pH 2, 25°C).

● chloramine; Δ free chlorine; ○ total active chlorine.

The relatively lower rate of chlorination of cystine at pH 8 than that at pH 2 may be ascribed partly to the formation of oxidation cystine intermediates.

The role of the amino groups in the chlorination reactions of cystine has also been studied using the *N,N'*-diacetyl cystine (Fig. 7). It is apparent that there is a gradual decrease in the free chlorine content of the reaction medium, accompanied by a gradual increase of the amount of *N*-chloro-*N,N'*-diacetyl derivatives. The chlorine consumed for their oxidation increased also gradually but the rate is relatively low when compared with the rate of cystine oxidation (Fig. 6).

The simultaneous increase both in the chlorine consumption and chloramine content of the reaction solution may be ascribed to two reactions. The first (faster) reaction leads to the formation of a chloramine while the second (slower) one leads to oxidation products. It is apparent that the *N,N'*-dichloro derivative of *N,N'*-diacetyl cystine is more stable than that of cystine, but less stable than the corresponding glycine derivatives.

Chlorination of aspartic acid

Aspartic acid (10 mmoles) at pH 2 was treated with 10 or 20 mmoles of initial chlorine content (Fig. 8). An oxidation reaction accompanied with the formation of chloramines took place. When using 40 mmoles of chlorine per 10 mmoles of aspartic acid, formation of *N*-dichloro aspartic acid equivalent to about 18 mmoles of chlorine occurred at the moment of mixing the reactants. The results show a relatively rapid decrease in the chloramine content accompanied by a moderate increase in the free chlorine content in the first two minutes (Fig. 9). This may be explained by decomposition of the *N*-dichloro compound to give oxidation products and free chlorine.

The high rate of oxidation of aspartic acid with chlorine solution at pH 2 is ascribed to the high rates of chloramine formation and to those of the oxidation of reaction products.

At pH 8, aspartic acid is oxidized with chlorine at nearly the same rate as pH 2. Consumption of the available chlorine was nearly complete after about 40 minutes of reaction.

In these cases no free aspartic acid could be detected in the reaction mixture. A large amount of aldehyde, most probably acetaldehyde, was formed.

Chlorination of lysine

It can be seen that at pH 2, about 50% of the available chlorine is consumed in few minutes (Fig. 10). We have found that in the reaction solution the higher amount of the unconsumed chlorine was present in the free state beside a relatively small amount of *N*-chloro derivatives. The maximum of *N*-chloro compounds equivalent to 10 mmoles of chlorine is produced only on using 40 mmoles of chlorine per 10 mmoles of lysine; the result which was not expected with a diamino compound. This may be attributed to the reaction of amino groups with the mineral acid in solution to give $-\text{NH}_3^+$ ion which evidently does not react with chlorine. It is apparent that the formed chloramine decreased rapidly, while free chlorine content decreased only slightly what can indicate that the oxidation reaction in the first minutes takes place mainly at the expense of the formed chloramine. The disappearance of chlor-

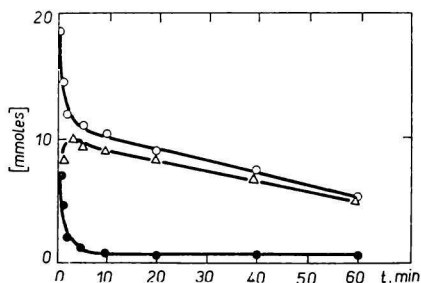


Fig. 10. The state and content of available chlorine (mmoles) during chlorination of 10 mmoles of lysine (20 mmoles of chlorine, pH 2, 25°C).

● chloramine; Δ free chlorine; \circ total active chlorine.

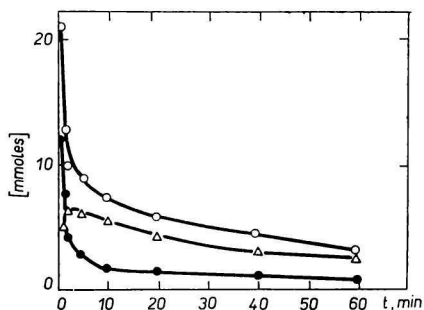


Fig. 11. The state and content of available chlorine (mmoles) during chlorination of 10 mmoles of arginine (20 mmoles of chlorine, pH 2, 25°C).

● chloramine; Δ free chlorine; \circ total active chlorine.

amine derivatives may be ascribed to their consumption at a rate higher than that of their formation. Thus the reaction rate is mainly controlled by the formation of such derivatives.

Chromatographic analysis indicated a complete removal of lysine from solution in the case of 40 mmoles of chlorine at pH 2 after one hour.

Chlorination of arginine

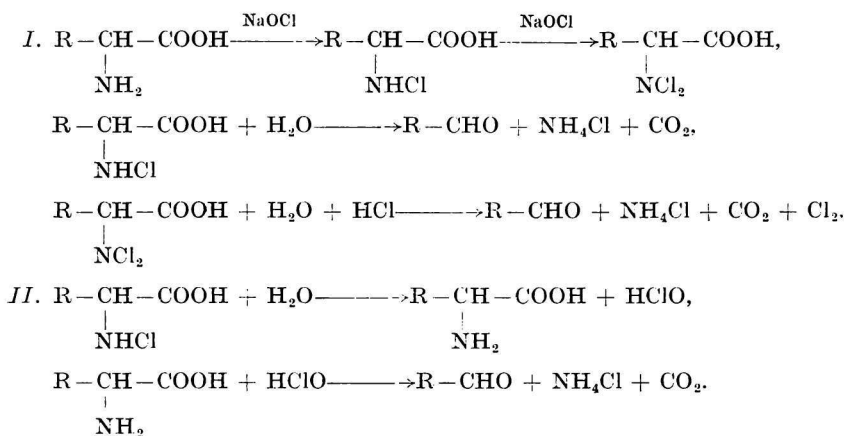
The reaction of arginine with chlorine brings about chloramine derivatives. The formed chloramine decreased gradually at the same rate as the chlorine consumption (Fig. 11). This indicates that the arginine oxidation takes place mainly through the formation of *N*-chloro and *N*-dichloro derivatives. The increase in the free chlorine content may be explained in the same way as in the case of previously studied amino acids. Comparison of the rate of oxidation of lysine and that of arginine shows that the latter is more reactive than the former.

Summing up, the action of chlorine on most of the studied amino acids is relatively higher at pH 8 than at pH 2. At pH 8, amino acids are mainly oxidized by hypochlorite ions present.

At pH 2, the first action of chlorine on the amino acid is in most cases a chlorination process either on the amino groups or on the aromatic nuclei. This process, in most cases, is completed in few seconds. Oxidation of amino acids at pH 2 takes place mainly through the formation of intermediate *N*-chloro derivatives. The rate of reaction, in this case, is controlled by the oxidative power of the formed chloramine and/or its ability to produce hypochlorous acid in aqueous solution.

Formation of stable chloro compounds as in the case of glycine influences greatly its rate of oxidation and a considerable part of this derivative is found in the reaction medium after one hour. Aspartic acid, cystine and arginine give *N*-chloro derivatives which decompose readily in solution. It is obvious that accumulation of *N*-chloro derivatives in the reaction solution generally accompanies lower rates of oxidation. This is the case with glycine, *N*-acetylglycine and *N,N'*-diacetyl cystine.

From the results obtained by the chlorination of amino acids studied, the following general reaction scheme can be suggested:



The apparent increase in free chlorine content of the reaction mixture at the expense of the chloramine content after commencing the reaction, provides a support for this suggested mechanism. Substitution in the amino groups by an acetyl group impedes to a great extent the formation of *N*-dichloro derivatives. Decrease of the oxidation rate of such substituted derivatives with chlorine solution gives evidence for the role of *N*-dichloro derivatives in oxidation reaction and provides an additional support to the suggested mechanism. Oxidation of cystine takes place mainly at the disulfide groups. Such oxidation does not exclude attack of the carbon atom in the molecule. The disulfide is most probably oxidized by the formed *N*-chloro derivatives of cystine either directly or through the formation of hypochlorous acid. Such derivatives are very unstable, so that small amounts are detected in the reaction mixture only when using high chlorine concentrations.

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