«-Amino Acids Chlorination in Aqueous Media

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Abstract: The reaction of chlorination of α -amino acids for 6 < pH < 11 has been studied. The reaction is an aliphatic electrophilic substitution, the rate determining step being the transfer of the chlorine atom between the HOCl oxygen and the nitrogen of the α -amino acid free amino group.

INTRODUCTION

As part of more extensive research on the reactivity of the N-halo derivatives, the α -amino acids chlorination reaction in aqueous media has been studied.

The α -amino acids react with chlorinating agents, yielding the corresponding N-Cl- α -amino acids, compounds of well known environmental and synthetic interest^{1,2}.

EXPERIMENTAL DETAILS

Aqueous solutions of chlorine have been used as chlorinating agent, having been prepared from a stock solution of sodium hypochlorite. All the other chemicals were $Merck^{(0)}$ p.a., with exception of the (L)-Proline, which was Sigma⁽⁰⁾. $H_2PO_4^-$ / NaOH, $H_3BO_3^-$ / NaOH and HCO_3^- / CO_3^{-2} aqueous mixtures were used as buffers.

The reactions were followed measuring the increase in the absorption at 255 nm due to the N-Cl- α -amino acid -267 nm in the case of the Sarcosine-, as well as the disappearance of the chlorinating agent at 292 nm. A SF-61 Hi-Tech Scientific[®] stopped-flow spectrophotometer thermostated by water flow to within ± 0.1 K was used.

For the pH measurements a combined glass electrode (calibrated with phthalate -pH = 4.00 ± 0.01 at 298 K- and phosphate -pH = 7.00 ± 0.01 at 298 K- buffers) and a Crison[®] 506 pHmeter were used.

Buffered solutions of α -amino acid and chlorinating agent at the same pH were used. Having into account that: a) at the end of the reaction the absorbance at 292 nm had completely vanished and b) no relevant signal was observed at the wavelengths corresponding to the (N,N)-di-Cl- α -amino

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acids absorption, it can be ruled out that the yield of N-Cl- α -amino acids is quantitative.

The corresponding kinetic equation was fitted to the experimental data by means of the Davies-Swann-Campey³ algorithm, as well as by the Marquardt one⁴. The values reported for the observed rate constants are an average of those obtained for at least five experiments.

RESULTS AND DISCUSSION

The reaction is a second order one, one order in relation to both the chlorinating agent and the α -amino acid.

 $r = k_{obs}$ [chlorinating agent] [α -amino acid] [1]

The rate constant has been proved to remain constant when varying the ratio $\frac{[\alpha-amino\ acid]}{[chlorinating\ agent]}$ as shown in Table 1. In this case, there are little unavoidable pH changes which affect the rate constant.

Table 1	L:	Rate	Constant	Dependence	on	Reactants	Ratio
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[Chlorinating agent] ^b	[Isoleucine] ^b	Hq	$k_{obs} * 10^{-4} / mol^{-1} dm^3 s^{-1}$
8.16*10 ⁻⁴	8.2*10 ⁻⁴	10.06	6.8 ± 0.3
8.16*10 ⁻⁴	1.6*10 ⁻³	10.01	7.6 ± 0.2
8.16*10 ⁻⁴	3.2*10 ⁻³	9.99	7.2 ± 0.2

^b Units: (mol dm⁻³)





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The rate constant depends strongly on the pH, passing through a maximum as demonstrated by Figure 1.

The observed rate constants and the corresponding pH values are shown in Table 2.

Amino acid	рН	$k_{obs} * 10^{-4} / mol^{-1} dm^3 s^{-1}$
	6.02	0.78 ± 0.03
	7.05	4.32 ± 0.06
	7.71	9.78 ± 0.08
Alanine	8.90	14.6 ± 0.2
ATUITINE	9.29	13.0 ± 0.4
	9.97	6.0 ± 0.1
	10.77	4.82 ± 0.06
	6.03	0.83 ± 0.02
	7.02	5.17 ± 0.07
	7.71	9.7 ± 0.2
2-amino butiric acid	8.90	13.9 ± 0.5
	9.29	13.1 ± 0.3
	9.97	7.1 ± 0.2
	10.77	4.27 ± 0.09
	6.03	0.89 ± 0.01
	7.00	5.4 ± 0.2
	7.69	9.5 ± 0.1
2-amino pentanoic acid	8.94	13.4 ± 0.7
	9.28	11.0 ± 0.2
	9.98	6.0 ± 0.2
	10.77	2.69 ± 0.03
	6.04	0.77 ± 0.02
	7.03	4.59 ± 0.04
	7.71	10.8 ± 0.4
2-amino bexanoic acid	8.94	12.9 ± 0.3
	9.21	10.4 ± 0.3
	9.98	5.0 ± 0.2
	10.77	2.33 ± 0.06

Table 2: Rate Constant Dependence with pH^a

a [α-amino acid] ≃ $1.2*10^{-3}$ mol dm⁻³; [chlorinating agent] ≃ $1.2*10^{-3}$ mol dm⁻³; T ≃ 298.0 K

Table 2 (Continued)

Amino acid	рН	$k_{obs} * 10^{-4} / mol^{-1} dm^3 s^{-1}$
	6.04	0.698 ± 0.009
	7.03	3.84 ± 0.06
	7.69	8.8 ± 0.2
Sarcosine	8.94	15.0 ± 0.8
	9.22	12.1 ± 0.4
	9.98	10.5 ± 0.1
	10.74	4.3 ± 0.3
	5.97	0.92 ± 0.03
	7.05	5.33 ± 0.07
	7.76	10.0 ± 0.2
Isoleucine	8.99	13.1 ± 0.4
	9.18	13.0 ± 0.5
	9.92	7.7 ± 0.3
	10.80	2.06 ± 0.02
	6.09	0.33 ± 0.01
	6.68	0.91 ± 0.02
	7.75	4.2 ± 0.1
Proline	9.02	6.29 ± 0.05
	9.33	4.88 ± 0.07
	10.07	3.2 ± 0.1
	10.80	3.2 ± 0.2
	6.04	0.36 ± 0.03
	7.04	1.33 ± 0.07
	7.68	3.14 ± 0.02
Amino isobutiric acid	8.94	4.78 ± 0.09
	9.23	5.9 ± 0.1
	9.96	4.1 ± 0.1
	10.75	1.67 ± 0.04
	6.10	1.28 ± 0.02
	7.04	6.35 ± 0.07
	7.70	12.6 ± 0.4
Glycine	8.86	18.8 ± 0.7
	9.26	25 ± 2
	9.97	19.7 ± 0.4
	10.76	4.1 ± 0.2

For the range 6 < pH < 11 it is found that:

$$k_{obs} = a \left(\frac{[H^{\dagger}]}{(b+[H^{\dagger}])(c+[H^{\dagger}])} \right)$$
[2]

where a, b, and c are empiric parameters.

In the working conditions, the hypochlorous acid and the hypochlorite ion $\operatorname{coexist}^5$:

HOC1
$$\longleftrightarrow$$
 C10 ^{Θ} + H ^{Θ} pK \simeq 7.537 (298 K) [3]

while the amino acids show two macroscopic acid-base equilibria $-(K_a)_1$ and $(K_a)_2$, each of them including two microscopic acid-base equilibria- and a tautomeric one.



The tautomerism constant K_r is of the 10⁵ order for amino acids such as those considered here, the equilibrium being displaced towards the zwitterion species⁶.

As a starting point it can be put forward that in the slow step the species depicted in equations [3] and [4] will take part, so that eight different processes could be considered.

The participation of the species:



can be rejected given that its low concentration would imply a bimolecular rate constant higher than the diffusion control limit.

On the basis of the dependency observed for the rate constants with the pH (equation [2]), four of the remaining processes can also be rejected.

Hence, the only reasonable determining steps to consider are those which correspond to the following bimolecular reactions:

$$\begin{array}{c} R^{2} \\ R^{1} - C - COO^{\Theta} + OC1^{\Theta} \\ \stackrel{\mu}{\longrightarrow} R^{1} - C - COO^{\Theta} + H_{2}O \\ R^{3} \stackrel{\mu}{\longrightarrow} H \\ R^{3} \stackrel{\mu}{\longrightarrow} R^{1} - C - COO^{\Theta} + H_{2}O$$
 [5]

$$R^{2} \xrightarrow{R^{2}} R^{1} \xrightarrow{k_{5}} R^{1} \xrightarrow{k_{7}} C_{1} \xrightarrow{k_{9}} R^{2} \xrightarrow{R^{2}} R^{1} \xrightarrow{k_{9}} R^{1$$

which are, from a kinetic point of view, indistinguishable.

Alternative elementary processes can be put forward which involve other chlorinating agents^{7,8}, however they would show k_{obs} versus pH dependencies different from those observed.

As a result, the following equation can be deduced:

$$k_{obs} = k K \left(\frac{[H^{+}]}{((K_{a})_{2} + [H^{+}])(K_{c} + [H^{+}])} \right)$$
[7]

where:

k is the second order observed rate constant
k is the rate constant for the elementary reaction,

 $i.e.: k_4 \text{ or } k_5$

K can be K_c or $(K_a)_2$, according to whether it is considered that the rate determining step is equation [5] or [6].

The maximum of the k_{obs} profiles *versus* the pH corresponds to the arithmetic mean of the pK values of the chlorinating agent and of the α -amino acid, as can be deduced from equation [7].

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Although steps [5] and [6] are indistinguishable from the kinetic point of view, reasons exist which permit us to lean towards the second.

Thus, in a process in fact similar as it is the nitrosation of amino acids, the participation of the free amino group in the rate determining step has been proved⁹.

On the other hand, it is difficult to imagine a reasonable bond making / bond breaking sequence for the direct transfer of the chlorine atom from the hypochlorite ion to the positively charged nitrogen of the amino group.

Obviously, the possibility exists that in the encounter pair the proton transfer takes place from the zwitterion amino group to the hypochlorite ion -an energetically unfavorable process- and, later on, the attack of the nitrogen electron pair on the chlorine atom, that is:



In general the proton transfer processes between nitrogen and oxygen are fast⁶, thus coming to the conclusion that what really controls the chlorination rate is the chlorine transfer between the HOCl oxygen and the nitrogen of the free amino group of the α -amino acid.

This conclusion reached by considering equation [5] as the rate determining step is essentially the same inferred from equation [6].

Finally, accepting that for the pH range from 6 to 11 the α -amino acids chlorination takes place as equation [6] indicates, the bimolecular rate constants shown in Table 3 result, all of them having the same magnitude order. The rates thus obtained for Glycine and Alanine agrees with the values found by Margerum et al¹⁰.

Amino acid	(pK _a) ^b	$k_{5}^{*10^{-7}} / \text{mol}^{-1} \text{dm}^{3} \text{s}^{-1}$
Isoleucine	9.76	6.4
Glycine	9.78	11.3
2-amino pentanoic acid	9.81	5.8
2-amino butiric acid	9.83	8.0
2-amino hexanoic acid	9.83	3.2
Alanine	9.87	3.4
Sarcosine	10.20	10.8
Amino isobutiric acid	10.21	3.5
Proline	10.65	2.0

Table 3: Bimolecular Rate Constants^a

* [α -amino acid] \approx 1.2*10⁻³ mol dm⁻³; [chlorinating agent] \approx 1.2*10⁻³ mol dm⁻³; T \approx 298.0 K

^b pK values for the amino acids taken from Christensen¹¹.

No structure for the transition state has been put forward as various possibilities exist, including the participation of at least one water molecule¹²:



The values of b and c parameters of equation 2 agrees satisfactorily with the corresponding values of K_c and $(K_a)_2$ for the hypochlorous acid and the α -amino acid^{11,13}.

The bimolecular rate values for the amino acids chlorination by HOCl are one magnitude order minor than in the chlorination by Cl_2 , the latter case being accepted as a diffusion controlled process¹⁰.

Considering the results shown in Table 3, the existence of a relationship between the basicity of the amino group of the amino acids and their bimolecular reaction rate with the HOCl does not seem to bear out.

At present, the processes of bromination and iodination of α -amino acids are being studied.

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