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KINETIC STUDY OF THE CHLORINE TRANSFER FROM *N*-CHLOROSUCCINIMIDE TO AMINO COMPOUNDS

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A kinetic study of the reactions of *N*-chlorosuccinimide (NCS) with glycine (Gly), sarcosine (Sar), 2-methylalanine (2MA), proline (Pro) and pyrrolidine (Pyr) was carried out. The reactions were found to be first order with respect to both NCS and the amine or amino acid and order −1 in proton concentration. In order to calculate the experimental activation parameters, the effect of temperature on the reaction rates was studied. The ionic strength and buffer concentration were found to have no effect on the rate constant. A reaction mechanism involving Cl^+ transfer from NCS to the amine or amino acid to form an *N*-chloro compound is proposed. © 1997 John Wiley & Sons, Ltd.

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INTRODUCTION

Nitrogen-containing organic compounds can be oxidized to *N*-chloro compounds by a wide variety of chlorinating agents, including *N*-chlorosuccinimide (NCS). Because the *N*-chloro compounds are themselves chlorinating agents, Higuchi *et al*.[1] classified them according to their capacity to transfer Cl^+ ions to another substrate.

One of the most salient features of NCS is its instability in aqueous solution, possibly due to its photochemical decomposition via radical intermediates.[2] NCS has been used as a halogenating reagent in various studies in aqueous solution. Thus, Higuchi and Hasegawa[3] studied the formation of dimethylchloramine by using the initial-rate method. They postulated two potential reaction pathways, even though the experimental results suggested that the process consisted of a Cl^+ ion transfer.

Recent kinetic studies on the oxidation reactions of amino acids with NCS and NBS (*N*-bromosuccinimide)[4, 5] have led to the proposal of a reaction mechanism in which an acyl hypohalite forms initially, and then decomposes to an aldehyde. This behaviour departs markedly from that of similar reactions where sodium hypochlorite was used as the halogenating reagent.[6, 7]

In order to clarify the mechanism of this type of reaction, in this work we carried out a kinetic study of the formation of *N*-chloro compounds by use of NCS as the halogenating reagent.

EXPERIMENTAL

Amino acid and amine solutions were prepared by direct weighing of commercial products: glycine (Carlo Erba), sarcosine and 2-methylalanine (Merck), proline (Sigma) and pyrrolidine (Aldrich). Acetic acid–sodium acetate, sodium dihydrogenphosphate–disodium hydrogenphosphate and boric acid–sodium borate buffers were made by direct weighing from acetic acid, sodium dihydrogenphosphate and boric acid (all Merck p.a. grade chemicals), respectively, and addition of an appropriate volume of sodium hydroxide solution to obtain the desired pH. The ionic strength was kept constant at $I=0{\cdot}5$ by adding $NaClO_4$.

Succinimide (SI) and NCS were purified by recrystallization[8] prior to use. NCS was freshly prepared and stored in a UV-opaque flask in order to avoid decomposition.

Because the reactions were very fast, they were monitored by using an Applied Photophysics stopped-flow spectrophotometer. The temperature of the solutions was kept constant at $25\pm0{\cdot}1$ °C by means of water circulating via a thermostated bath. The equipment was furnished with two syringes that were used to inject the amine or amino acid and the NCS solution at the required pH, respectively.

On mixing the amine or amino acid solution with the NCS solution, an absorption band with a maximum at about 260 nm, typical of *N*-chloro compounds,[9, 10] was observed. Figure 1 shows the reaction spectrum for *N*-chloroglycine formation.

The reactions were studied in the dynamic spectrophotometric mode; absorbance values were recorded at the

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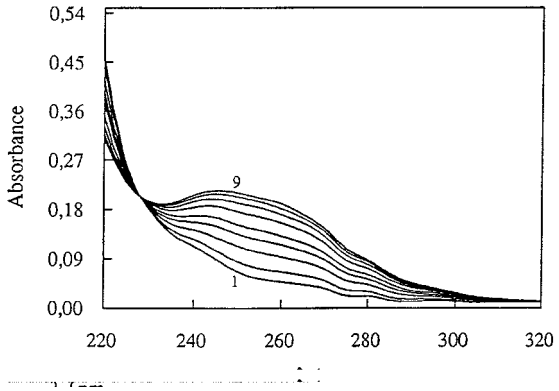


Figure 1. Spectra for *N*-chloroglycine formation. $T=25$ °C, $[NCS]=6\times10^{-4}$ M, $[Gly]=6\times10^{-3}$ M, pH = 6·8, $\Delta t=12$ ms

wavelength of the absorption maximum for the *N*-chloro compound formed in each case.

The reactions were studied using the isolation method, the amino acid being present in at least a tenfold excess over the NCS. Absorbance–time data were fitted by first-order integrated rate expressions and the slope was used to calculate the pseudo-first-order rate constant.

After the reaction order had been determined, the influence of the reagent concentrations and the different experimental variables affecting the reaction medium (temperature, buffer concentration, ionic strength and pH) were investigated.

pH measurements were made with a Radiometer PHM82 pH meter that was calibrated with commercially available buffer solutions of pH 4·01, 7·00 and 10·02.

RESULTS

A preliminary batch of experiments was conducted at different NCS concentrations and constant concentrations of all other reactants. The observed pseudo-first-order rate constants given represent the means of 5–10 kinetic runs.

Table 1 shows the rate constants obtained for the reaction with *N*-chloroglycine over the NCS concentration range studied. Based on these results, the initial NCS concentration can be assumed not to influence the rate constant, which confirms that the reaction is first order in this reagent.

Table 1. Influence of initial concentration on the formation rate constant for *N*-chloroglycine, with $[Gly]=3\cdot0\times10^{-2}$ M, pH = 6·80, $I=0\cdot5$ ($NaClO_4$), $T=25$ °C

[NCS] (M)	k_{obs} (s^{-1})
$3\cdot0\times10^{-4}$	9·33 ± 0·20
$7\cdot5\times10^{-4}$	9·45 ± 0·22
$1\cdot5\times10^{-3}$	9·30 ± 0·25
$2\cdot0\times10^{-3}$	9·23 ± 0·20
$2\cdot75\times10^{-3}$	9·47 ± 0·22

Table 2. Influence of glycine concentration on the formation rate constant for *N*-chloroglycine, with $[NCS]=5\times10^{-4}$ M, pH = 6·80, $I=0\cdot5$ ($NaClO_4$), $T=25$ °C

[Gly] (M)	k_{obs} (s^{-1})
$5\cdot0\times10^{-3}$	2·03 ± 0·04
$1\cdot5\times10^{-2}$	6·04 ± 0·08
$2\cdot5\times10^{-2}$	10·09 ± 0·20
$3\cdot5\times10^{-2}$	13·86 ± 0·20
$4\cdot5\times10^{-2}$	17·43 ± 0·40

The influence of the initial concentration of amine or amino acid was studied by varying it while keeping constant those of the other reactants. Table 2 gives the results obtained for *N*-chloroglycine. The plot of log k_{obs} against log [Gly] was a straight line of slope 0·98 ± 0·01, so the reaction order with respect to glycine was unity.

The influence of the proton concentration on the formation rate constant for *N*-chloroglycine at constant ionic strength $I=0\cdot5$ was studied by using buffer solutions of acetic acid–sodium acetate, sodium dihydrogenphosphate–disodium hydrogenphosphate and boric acid–sodium borate. The results are shown in Table 3. A plot of log k_{obs} vs pH was a straight line of slope 0·94 ± 0·01, so the rate constant was inversely proportional to the proton concentration for the *N*-chloro compounds studied.

The effect of the buffer concentration on the reaction rate was studied using acetic acid–acetate buffer solutions of different concentrations. The rate constant did not vary with the buffer concentration, and was also found to be independent of the nature of the buffer.

We also studied the influence of the ionic strength on the observed rate constant by varying the concentration of $NaClO_4$ with constancy of all other experimental parameters. The reaction rate was found to be independent of ionic strength in the range studied (0·03–0·5 M).

In order to calculate the experimental activation parameters for the formation of the *N*-chloro compounds, the influence of temperature on the reaction was investigated. Figure 2 shows the results obtained for *N*-chloroglycine and *N*-chloropyrrolidine, which conformed to the Arrhenius equation and the theory of absolute rates, and were used to calculate the activation energy (E_a), enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) for the *N*-chloro compounds studied.

MECHANISM AND DISCUSSION

Two mechanisms have been postulated for the reaction of NCS, with (a) nucleophilic attack on the chlorine atom of the *N*-chlorosuccinimide by the unprotonated amino nitrogen and (b) the other, previously reported,[4,5] involving nucleophilic attack of the carboxyl group of the amino acid on the chlorine atom to yield an acyl hypochlorite (Scheme 1).

Both are in keeping with the experimental observation

Table 3. Influence of proton concentration on the formation rate constant for *N*-chloroglycine, with [NCS] = 5×10^{-4} M, [Gly] = 5×10^{-3} M, I = 0·5 ($NaClO_4$), T = 25 °C

pH	k_{obs} (s^{-1})	pH	k_{obs} (s^{-1})
Boric acid–borate:		Acetic acid–acetate:	
8·90	182 ± 2	5·41	0·0823 ± 0·005
8·60	90 ± 1	4·98	0·030 ± 0·001
8·11	28·6 ± 0·3	4·42	$9\cdot1\times10^{-3}\pm5\times10^{-4}$
		3·86	$3\cdot8\times10^{-3}\pm0\cdot2\times10^{-4}$
Dihydrogenphosphate–hydrogenphosphate:			
7·47	8·5 ± 0·2		
7·09	3·62 ± 0·04		
6·36	0·66 ± 0·01		

that the rate of reaction was independent of ionic strength, since this suggests that at least one of the reactants in the rate-controlling step is electrically neutral.

In order to account for the experimental behaviour observed, and taking into account the potential species and equilibria involved, the mechanism depicted in Scheme 2 is proposed, according to which the amino acid, with an unprotonated amino group, reacts with NCS to give an *N*-chloroamino acid.

Because the reaction takes place via the amino acid with an unprotonated amino group, the only reactive species present in the reaction medium will be C and D. Hence the reaction rate will be given by

$$v = k_2[D][NCS] + k_3[C][NCS]$$

The concentrations of C and D can thus be expressed as functions of the acidity and the total amino acid (Aa) concentration:

$$[C] = K_I K_{II}[Aa]/([H^+]^2 + K_I[H^+] + K_I K_{II})$$
$$[D] = K_e[Aa][H^+]/([H^+]^2 + K_I[H^+] + K_I K_{II})$$

The first step of the process involves the amino acid protonation equilibria, where K_I and K_{II} are the macroscopic equilibrium constants for the loss of the first and second proton, respectively. The values of the microscopic constants can be estimated from K_I and K_{II} by assuming the microscopic constant K_I' to be equal to the acidity constant for the corresponding amino acid ester (K_e).[11] Also, since $K_I \gg [H^+] \gg K_{II}$, under the conditions used in this work, these expressions may be simplified to

$$[C] = K_{II}[Aa]/[H^+]$$
$$[D] = K_e[Aa]/K_I$$

so that the rate law may be written as

$$v = (k_2K_e[Aa]/K_I + k_3K_{II}[Aa]/[H^+])[NCS]$$

and the first-order pseudo-constant is given by

$$k_{obs} = k_2K_e[Aa]/K_I + k_3K_{II}[Aa]/[H^+]$$

Therefore, the reaction is first order with respect to the total amino acid concentration, consistent with experimental evidence, and the rate constant is linearly dependent on the reciprocal of the proton concentration, with slope $k_3K_{II}[Aa]$ and intercept on the ordinate $k_2K_e[Aa]/K_I$. Our experimental results lead to a straight line without a significant intercept, indicating that the term containing k_2 is much smaller than that containing k_3 and that the reaction takes place through species C preferentially. Therefore, the expression for observed rate constant will be given by

$$k_{obs} = k_3K_{II}[Aa]/[H^+]$$

This equation can be used to calculate the second-order

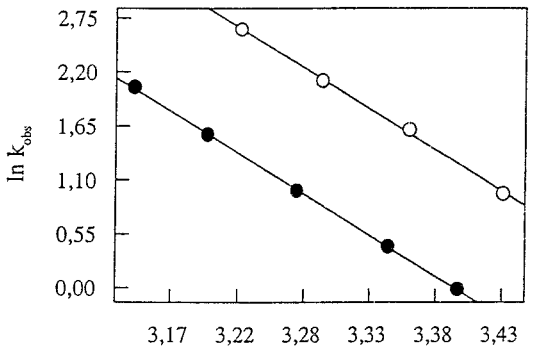
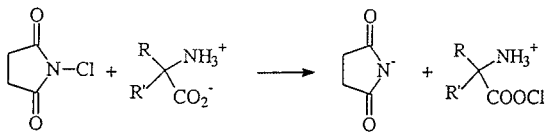
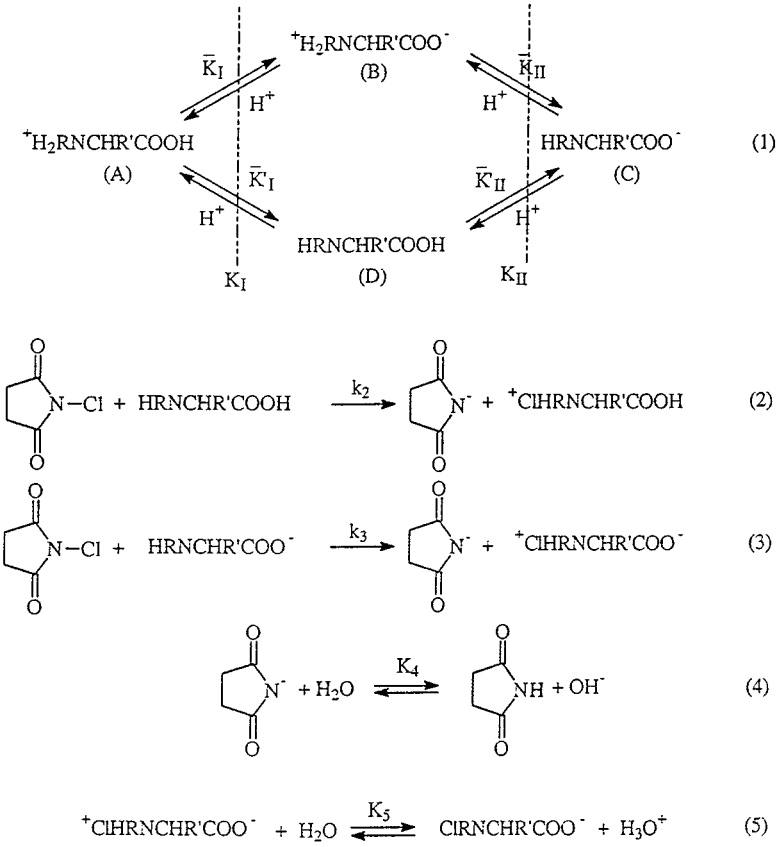


Figure 2. Influence of temperature on the formation rate constant for (●) *N*-chloroglycine (T = 21·4, 26·1, 32·7, 39·6, 45·1 °C) and (○) *N*-chloropyrrolidine (T = 18·3, 24·7, 31·0, 37·0 °C). [NCS] = 5×10^{-4} M, [Gly] = [Pyr] = 5×10^{-3} M, pH = 6·87, I = 0·5 ($NaClO_4$)



Scheme 1



Scheme 2

rate constant, k_3. A plot of $k_{obs}[H^+]$ against the amino acid concentration should be a straight line with zero intercept, the slope of which can be used to calculate k_3 provided pK_{II} is known[12] at the ionic strength used (I=0·5). Table 4 shows the k_3 values calculated for the different *N*-chloro compounds studied.

The alternative mechanism (chlorination of the carboxylate group) also leads to the prediction that k_{obs} is proportional to $[Aa]/[H^+]$. However, the correlation between k_3 and the pK_a of the amino compound (slope = 0·9 ± 0·1; Figure 3), and in particular the satisfaction of the correlation by pyrrolidine, which has no carboxyl group, rules out the formation of an acyl hyopchlorite intermediate. Moreover, if COO^- were the active form, a pH-dependent region (at pH close to pK_a of the COO^- group of the amino acid) should be expected, contrary to the experimental behaviour.

Table 4. Values of pK_{II} and formation rate constants (k_3) for the *N*-chloro compounds studied

Amino acid	k_3 (l mol^{-1} s^{-1})	pK_{II} (25 °C, I=0·5)[12]
Gly	(6·3 ± 0·4) × 10^5	9·778
2MA	(1·29 ± 0·04) × 10^6	10·10
Sar	(2·09 ± 0·06) × 10^6	10·20
Pro	(5·7 ± 0·1) × 10^6	10·64
Pyr	(1·55 ± 0·04) × 10^7	11·31

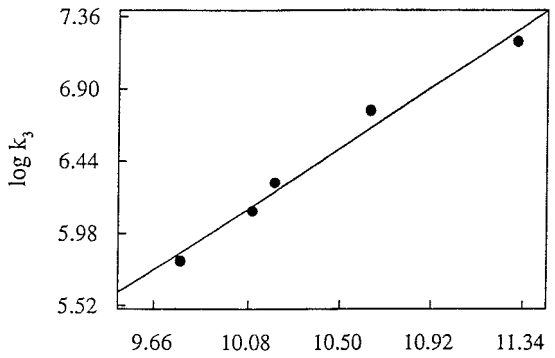


Figure 3. Correlation between the *N*-chlorination rate constant, k_3, and pK_a values for the nitrogen-containing compounds studied

Table 5. k_3(Br)/k_3(Cl) ratios obtained for the reactions of NBS and NCS with the different compounds studied

Amino acid	k_3 (Br)[15]	k_3(Br)/k_3(Cl)
Gly	$2{\cdot}7\times10^6$	$4{\cdot}3\pm0{\cdot}3$
2MA	$11{\cdot}8\times10^6$	$9{\cdot}1\pm0{\cdot}4$
Sar	$3{\cdot}56\times10^6$	$1{\cdot}8\pm0{\cdot}1$
Pro	$15{\cdot}5\times10^6$	$2{\cdot}7\pm0{\cdot}1$
Pyr	$47{\cdot}4\times10^6$	$3{\cdot}0\pm0{\cdot}1$

Table 6. Influence of succinimide concentration on the formation rate constant for *N*-chloroglycine. [NCS] = 5×10^{-4} M, [Gly] = $2{\cdot}5\times10^{-2}$ M, pH = 6·70, I = 0·5 ($NaClO_4$)

[SI] (M)	k_{obs} (s^{-1})
0·025	$7{\cdot}50\pm0{\cdot}10$
0·050	$7{\cdot}60\pm0{\cdot}12$
0·100	$7{\cdot}57\pm0{\cdot}11$
0·150	$7{\cdot}45\pm0{\cdot}10$
0·200	$7{\cdot}60\pm0{\cdot}10$

Similar results have been observed previously for the transfer of Br^+ from NBS to these amino compounds, the slope of the corresponding regression line being $0{\cdot}8\pm0{\cdot}1$.[13] Table 5 compares the values of k_3(Br) and k_3(CI) obtained for each amino compound under the same reaction conditions, and shows that bromonium ion transfer is faster than chloronium ion transfer. Excellent correlations between rate constants and nucleophile basicity have previously been reported for the *N*-halogenation of amines and amino acids by sodium hypochlorite[6, 7] or sodium hypobromite[14] and for several other chemically controlled reactions involving chloronium ion transfer, such as the reactions of chloramines with amines, amino acids and peptides[15, 16] and the oxidation of iodide,[17] bromide,[18] cyanide,[19] nitrite[20] or sulphite[21] by hypochlorous acid or *N*-chloramines, which involve nucleophilic attack on the chlorine, followed by cleavage of the Cl–O bond and the loss of an OH group. In every case where the reaction is not diffusion controlled, the rate constant increases with increasing basicity of the amino group of the substrate.

When the reaction is chemically controlled rather than diffusion controlled, the reactivity of a halogenating agent with a given nitrogenous compound is expected to be related directly to the electrophilicity of the halogenating agent.

N-Bromoamines form more rapidly than do the *N*-chloroamines. The rate constants for the reaction of NXS with amines and amino acids are 1·8–9·1 times greater for X = Br than for X = Cl. Such an order of reactivity of NBS and NCS is in the direction expected from the electrophilicities of NXS, which are in the order of NIS > NBS > NCS because of the increasing electronegativities and decreasing polarizabilities in the same order. In addition, the Br atom can better delocalize part of the total electron density of the negatively charge activated complex in its d orbitals than can the Cl atoms, thus providing the transition state with additional stability.

From the k_3(Br)/k_3(Cl) ratio of 1·8 to 9·1, we can say that the halogen dependence is not really high as compared with *N*-chloro- and *N*-bromoamine hydrolysis.[22] From the Brønsted plot (see Figure 3), $\beta = 0{\cdot}9\pm0{\cdot}1$ for NCS and $\beta = 0{\cdot}8\pm0{\cdot}1$ for NBS,[13] which suggests that there is nearly full bond formation between the amine and the halogen in the transition state. According to a later S_N2-like transition state, a small halogen influence is observed.

The influence of succinimide concentration on the reaction rate constant was also investigated; as can be seen from Table 6, this variable had no appreciable effect. The study was carried out in phosphate buffer at pH 6·7 and a constant ionic strength I = 0·5. The lack of influence observed excludes a potential reversible character for the reaction.

The calculated k_3 values can be used to obtain the overall activation enthalpy and entropy (Table 7) for the process by using the following equation:

$$\ln\left(\frac{k_3K_{II}}{T}\right)=\ln\left(\frac{k_B}{h}\right)+\frac{\Delta S^{\neq}_{reac}}{R}-\frac{\Delta H^{\neq}_{reac}}{RT}$$

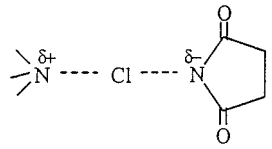
where

$$\Delta S^{\neq}_{reac}=\Delta S^{\circ}_{II}+\Delta S^{\neq}_{3}$$
$$\Delta H^{\neq}_{reac}=\Delta H^{\circ}_{II}+\Delta H^{\neq}_{3}$$

where ΔS°_{II} and ΔH°_{II}, the overall entropies and enthalpies for the equilibria between the neutral and anionic forms of the amino acid, are known.[12] Hence the activaction parameters

Table 7. Activation parameters for the formation of the *N*-chloro compounds studied, with $\Delta S^{\neq}$ values in J mol^{-1} K^{-1} and E_a and $\Delta H^{\neq}$ values in kJ mol^{-1}

Amino acid	$\Delta H^{\neq}_{reac}$	$\Delta S^{\neq}_{reac}$	ΔH°_{II}[12]	ΔS°_{II}[12]	$\Delta H^{\neq}_{3}$	$\Delta S^{\neq}_{3}$	E_a
Gly	$64{\cdot}7\pm0{\cdot}4$	-112 ± 1	44·4	−38·5	20	−74	$67{\cdot}4\pm0{\cdot}6$
2MA	74 ± 1	-86 ± 4	47·7	−35·1	26	−51	$76{\cdot}1\pm1{\cdot}1$
Sar	59 ± 1	-124 ± 3	41·0	−57·7	18	−66	$61{\cdot}5\pm1{\cdot}1$
Pro	$64{\cdot}4\pm0{\cdot}6$	-101 ± 2	43·3	−58·6	21	−42	$67{\cdot}0\pm0{\cdot}6$
Pyr							$66{\cdot}6\pm1{\cdot}7$



Scheme 3

$\Delta S_3^{\neq}$ and $\Delta H_3^{\neq}$ for step 3 in Scheme 2 can also be calculated (Table 7).

In conclusion, the *N*-chlorination of amines and amino acids by reaction with NCS takes place by a mechanism involving transfer of Cl^+ from NCS to the unprotonated nitrogen atom of the amino compound. This reaction falls into the general class of nucleophilic substitution reactions involving transfer of a cation such as NO^+,[23] Cl^+ [7] or Br^+;[14] for the reaction of NCS with amino compounds, the rate-controlling step can be considered as a concerted process in which the amino compound nucleophilically displaces the leaving group (the succinimide anion) of the halogenating agent. Since both NCS and the unprotonated amino group of the amino compound are electrically neutral, charge separation must take place in the transition state (Scheme 3), a hypothesis supported by the large negative entropies calculated for step 3, which suggest that the transition state is highly solvated.

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