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Kinetics and mechanism of base-catalysed hydrolysis of L-asparagine and its copper(II) complex

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Kinetic studies on hydrolysis of L-asparagine and Cu(II)-L-asparagine complex have been carried out in sodium hydroxide solution (0.1-2.5 mol dm^{-3}). The pseudo-first order rate constants follow the general relationship: $k_{obs} = k_0[OH^-]$ where k_0 is an empirical constant. The probable mechanism of hydrolysis involves monoanionic tetrahedral inter[illegible]. The effect of Cu(II) ion on hydrolysis [illegible]gine has been discussed and act[illegible]luated.

Leach and Lindley[1] studied the kinetics of acid hydrolysis of L-asparagine, L-asparaginylglycine and L-leucyl-L-asparagine and proposed a probable mechanism[1]. Hydrolysis of N-acylaspartic monoamides was carried out by Ali and Capindale[2] and they proposed mechanism to account for selective release of aspartic acid from proteins. The effect of Cu(II) ions on asparagine and isoasparagine hydrolysis was observed by Stromberg[3] in alkaline medium. When the rate of hydrolysis of asparagine was compared with that of its Cu(II) complex under low [hydroxide ion] (10^{-5} to 10^{-2} mol dm^{-3}), rate of hydrolysis of Cu(II) complex was found greater in $[OH^-]$ range 10^{-4} to 10^{-2} mol dm^{-3}. The effect of $[OH^-]$ was also studied for the hydrolysis of isoasparagine at higher $[OH^-]$. The rate of hydrolysis of isoasparagine Cu(II) complex decreased at 0.1 mol dm^{-3} $[OH^-]$. The mechanism of hydrolysis of L-asparagine and its Cu(II) complex was not discussed by Stromberg[3]. In continuation of our work[4,5] on alkaline hydrolysis of amides and related compounds, we report herein the kinetics and mechanism of hydrolysis of L-asparagine and its Cu(II) complex.

Experimental

L-Asparagine (Fluka) and cupric sulphate (AnalaR, BDH) were used as such. A concentrated stock solution of sodium hydroxide (E. Merck) of about 17 mol dm^{-3} was prepared. It was filtered to remove undissolved carbonate, diluted to prepare a ca. 3.5 mol dm^{-3} solution and standardization using standard HCl and potassium hydrogenphthalate. Cu(II)-L-asparagine complex was prepared in the reaction vessel by mixing L-asparagine, cupric sulphate and sodium hydroxide solutions. Kinetic measurements were carried out by monitoring the production of ammonia as a function of time by the method described earlier[4,5]. Solubility of Cu(II) in concentrated NaOH solution (1.0 mol dm^{-3}) was determined iodometrically and the formation constant of $Cu(OH)_4^{2-}$ was calculated.

Results and discussion

The rate of hydrolysis of L-asparagine in alkaline medium was independent of ionic strength (μ). To study the effect of temperature, the kinetic studies were carried out at different temperatures ranging from 45 to 90°C and various activation parameters were calculated by Arrhenius and Eyring equations. The values are $\Delta H^{\neq} = 43.40 \pm 1.20$ kJ mol^{-1}, $\Delta S^{\neq} = -190.01 \pm 4.2$ JK^{-1} mol^{-1} and $\Delta H^{\neq} = 49.80 \pm 1.10$ kJ mol^{-1}, $\Delta S^{\neq} = -171 \pm 1.17$ JK^{-1} mol^{-1} for L-asparagine and Cu(II)-L-asparagine complex respectively.

The effect of $[OH^-]$ was studied within the range of 0.1 to 2.5 mol dm^{-3} [NaOH] at 45, 55, 65 and 75°C by keeping ionic strength constant at 3.0 mol dm^{-3} with KNO_3 solution. The observed results fit the empirical Eq. (1).

$$k_{obs} = k_0[OH^-] \quad \ldots(1)$$

where k_0 is an empirical constant.

The effect of [Cu(II)] on the hydrolysis of L-asparagine was studied at different concentrations. These results are given in Fig. 1. The rate constants decreased with the addition of Cu(II) and became minimum at [Cu(II)] = [L-asparagine]. The variation of $[OH^-]$ was also studied on Cu(II)-L-asparagine complex (Fig. 2) and pseudo-first order rate constants were found to follow Eq. (1).

The alkaline hydrolysis of acyl derivatives is a two step reaction which proceeds by the formation of an unstable tetrahedral intermediate. In an extensive study of anilides[6-8], shifting of the order of

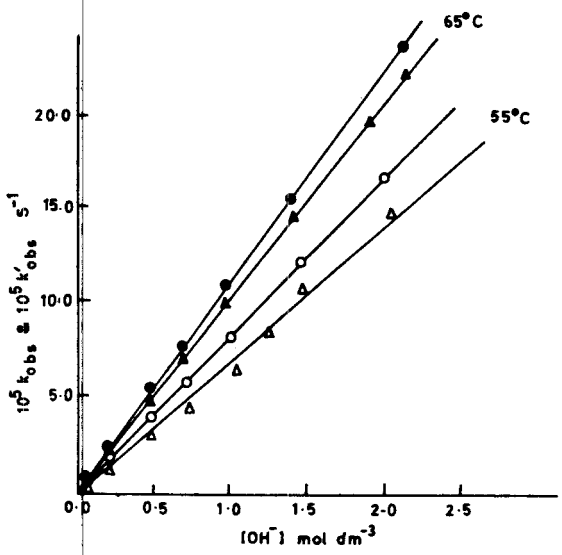


Fig. 1—Plots of variation of pseudo-first order rate constants with sodium hydroxide concentration for the hydrolysis of L-asparagine (○,●) and Cu(II)-L-asparagine (△,▲) [L-asparagine] = 0.005 M; [Cu(II)] = 0.005 M; ionic strength = 3.0 M.

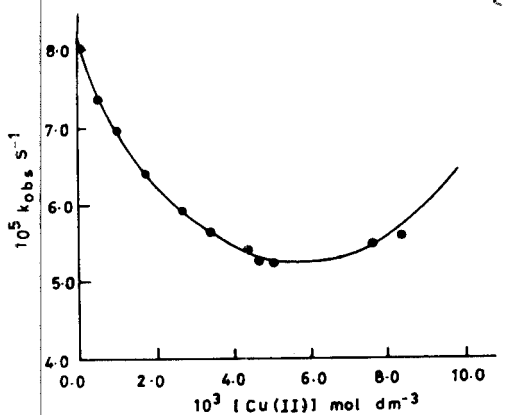


Fig. 2—Effect of variation of [Cu(II)] on the hydrolysis of L-asparagine at temp. = 348 K, [L-asparagine] = 0.005 M, [NaOH] = 1.0 M.

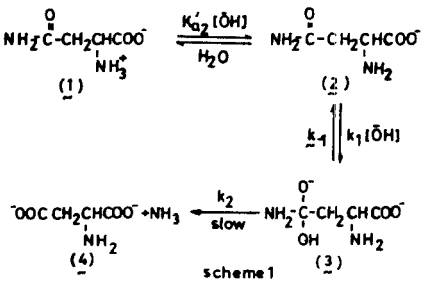
Table 1—The values of k_0 at different temperatures for the hydrolysis of L-asparagine and its Cu(II) complex

Conditions: [L-Asparagine] = 0.005 mol dm^{-3}; μ = 3.0 mol dm^{-3} and [Cu(II)] = 0.005 mol dm^{-3}

Temp. K	$10^4 k_0$ for L-asparagine ($dm^3 mol^{-1} s^{-1}$)	$10^4 k_0$ for Cu(II)-asparagine ($dm^3 mol^{-1} s^{-1}$)
318	0.04	0.04
328	1.19	1.20
338	1.25	1.35
348	1.66	1.70

the reaction from second to first order kinetics has been explained by the formation of a tetrahedral addition intermediate between a nucleophilic reagent and an acyl compound which then reacts further to expell the leaving group with the formation of products.

The simplest stepwise mechanism which could explain the observed results is shown in Scheme 1. Considering (3) as a transient chemical species and applying steady-state approximation, Eq. (2) is obtained.



scheme 1

$$k_{obs} = \frac{k_1 k_2 K'_{a2} [\bar{O}H]^2}{(k_{-1} + k_2)(1 + K'_{2a} [\bar{O}H])} \quad \ldots (2)$$

where $K'_{a2} = K_{a2} /$ [illegible]

$$K_{a2} = \frac{[^-OOCCH(NH_2)CH_2 \text{[illegible]}]}{[^-OOCCH(NH_3^+)\,CH_2CONH_2][H_2O]} \quad \ldots (2$$

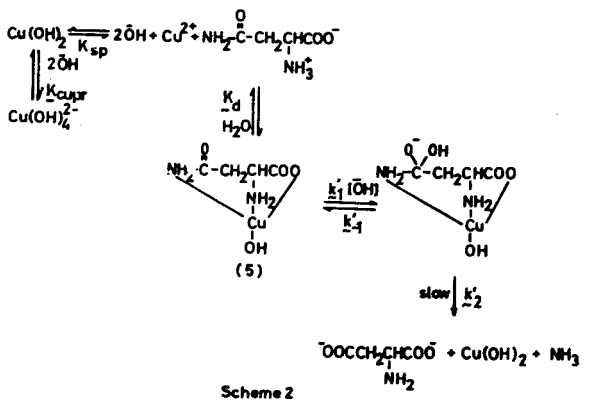
The value of pK_{a2} for L-asparagine is 8.80 at 50°C, therefore, $1 \ll K'_{a2}$ [$\bar{O}$H], then Eq. (2) reduces to Eq. (3)

$$k_{obs} = \frac{k_1 k_2 [OH^-]}{(k_{-1} + k_2)} \quad \ldots (3)$$

which is similar to Eq. (1) with $k_0 = k_1 k_2 / (k_{-1} + k_2)$. The values of k_0 at different temperatures are summarized in Table 1.

Here, only anionic form (**2**) of L-asparagine is reactive towards nucleophilic attack of the hydroxide ion at carbonyl carbon atom.

L-asparagine reacts with Cu(II) in alkaline medium to form a violet complex[9] and its structure (**5**) was proposed by the electrochemical studies where amide-nitrogen coordinates with the metal[10]. The mechanism of the alkaline hydrolysis of Cu(II)-L-asparagine complex is presented in Scheme 2. Equation (4) has been derived on the basis of the observed results and proposed mechanism.



Scheme 2

$$k'_{obs} = \frac{k_1' k_2' [OH^-](K_{cupr}[OH^-]^4 + K_{sp})}{(k'_{-1} + k_2'(K_{cupr}[OH^-]^4 + K_d[OH^-] + K_{sp})} \quad \ldots (4)$$

In Eq. (4), $K_{sp} = [Cu^{2+}][OH^-]^2$, $K_{cupr} = [Cu(OH)_4^{2-}]/[OH^-]^2$ and $K_d = [\text{L-asparagine}]\,[Cu(II)][OH^-]/[\text{Complex}]$. The value of K_d was reported[10] to be 2.51×10^{-14} mol^2 dm^{-6}, while the value of K_{cupr} has been determined to be 2.82×10^{-3} mol dm^{-3}. Since K_{sp} (2.2×10^{-20} mol^3 dm^{-9}) and $K_d[OH^-]$ are negligible in comparison with $K_{cupr}[OH^-]^4$, Eq. (4) reduces to Eq. (5).

$$k'_{obs} = \frac{k_1' k_2' [OH^-]}{(k'_{-1} + K'_2)} \quad \ldots (5)$$

The rate of hydrolysis of L-asparagine increased with the addition of Cu(II). At low [OH$^-$] (10^{-4} to 10^{-2} mol dm^{-3}), the formation of 2:1 complex with coordination of Cu with carbonyl oxygen of amide group is reasonable[3]. The comparison between the rate of hydrolysis of Cu(II) complexes of asparagine and isoasparagine, where isoasparagine complex hydrolysed more rapidly, the argument that coordination of carbonyl oxygen of amide group of asparagine with Cu(II) ion would produce rigidity and inhibit the attack of OH$^-$ is not valid in view of recent advancements[11-14] regarding metal ion catalysis of amide group hydrolysis. Hypothesis presented by Sayre[14] for metal ion catalysis of amide hydrolysis explained catalytic effect of metal ion in terms of enhancement of rate determining step which is the breakdown of the tetrahedral intermediate. The coordination of carbonyl group with Cu(II) cannot affect the rate of formation of tetrahedral intermediate but the rate of break down of this intermediate is enhanced. The attack of hydroxide ion becomes the rate determining step. Catalysis occurs by coordination of metal ion to the oxygen atom of the tetrahedral intermediate which greatly enhances its nucleophilicity and causing rapid cleavage of C−N bond. Therefore, metal coordination with carbonyl oxygen accelerates the rate of hydrolysis and coordination of amide nitrogen is responsible for the inhibition of rate of hydrolysis.

The enhancement of rate of hydrolysis of asparagine and isoasparagine complexes of Cu(II), as observed by Stromberg, can be explained in terms of coordination of carbonyl oxygen with the Cu(II). In high [OH$^-$] i.e. [OH] $\gg$ 0.1 mol dm^{-3}, the amide nitrogen is coordinated with Cu(II) in place of carbonyl oxygen which retards the rate of hydrolysis due to hindering the protonation of amide group in the tetrahedral intermediate.

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