Influence of Calcium Ions on the Mechanism of Oxime Formation from Acetoin

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The effect of calcium ions on the reaction of acetoin with hydroxylamine was studied. This reaction proceeds by a two-step mechanism: the attack of hydroxylamine on the carbonyl compound to give a tetrahedral addition intermediate, and the dehydration of this intermediate to form the oxime. The presence of calcium ions decreases the value of the equilibrium constant of the tetrahedral addition intermediate formation, but increases the overall rate constant of the reaction when dehydration is the rate-determining step. Evidence suggests that the effect of calcium ions is through the formation of a complex with the hydroxyl groups of the tetrahedral addition intermediate, facilitating the dehydration step of the reaction.

The role and importance of the calcium ion in many biological systems are well-known. Calcium ions are commonly bound to proteins through carbonyl groups. In addition to water, carboxylato and carbonyl groups, other kinds of ligands are involved in the calcium-binding sites of biological systems. Of particular importance in our case is the hydroxyl group, which interacts with calcium ions through the oxygen atom.[1)]

The importance of the calcium ion in biological systems can also be realized when it is understood that the carbohydrate binding to some family of proteins, such as the mammalian lectins, is strictly calcium dependent.[2)]

The calcium and other ions were shown to catalyze the nucleophilic reactions of acetyl phosphate[3)] and to stabilize Schiff-base formation.[4—8)] Some divalent metal ions (Pb^{2+}, Zn^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}) were found to be kinetically active in promoting the formation of *N*-salicylideneglycinate.[9)]

Our interest in certain biochemical processes, such as the Maillard reaction,[10,11)] and a consideration of the similarity of its initial steps to the reactions of oxime formation from hydroxylamine and carbonyl compounds, lead us to study the effect of calcium ions on the reaction of acetoin (3-hydroxy-2-butanone) — an intermediate of the Maillard reaction — with hydroxylamine.

Results and Discussion

It has been established that the formation of oximes,[12)] semicarbazones,[12,13)] thiosemicarbazones,[14)] phenylhydrazones,[15)] and Schiff bases[16—18)] occurs along with a rate-determining attack of the nucleophilic reagent on the carbonyl compound to form an addition intermediate under strong acid conditions. However, under neutral or basic conditions, dehydration of the tetrahedral addition intermediate becomes the rate-determining step. An inflexion in the pH-rate profile reflects the transition of the rate-determining step. The attack of the nucleophile on the carbonyl group can be uncatalyzed or oxonium ion-catalyzed, while dehydration can be acid-catalyzed, spontaneous and base-catalyzed.[19)]

The reaction of acetoin with hydroxylamine to form oxime was carried out in water at 25 °C, ionic strength 1.0 M (KCl) ($1\ \mathrm{M} = 1\ \mathrm{mol\,dm^{-3}}$). The concentration of hydroxylamine was in necessary excess so as to obtain pseudo-first-order reactions. The pH dependence of the observed second-order rate constants ($k_{2\mathrm{obs}}$), determined by extrapolation to zero buffer concentration in each pH, is shown in Fig. 1. The pH-rate profile can be classified as type C according to Sayer.[20)] This kind of profile is observed with amines of moderate basicity and a carbonyl compound having good equilibrium affinity with this amine. The profile exhibits only one change in the rate-determining step, which is the transition of an uncatalyzed attack (k_a) of the amine on the carbonyl compound, occurring between pH 0.5 and 1.0, to the oxonium ion-catalyzed dehydration of a tetrahedral addition intermediate (**I**), which proceeds between pH 1.0 and 7.5 ($k_{\mathrm{H^+}}$). From pH 7.5 to pH 9.0 the reaction proceeds by spontaneous dehydration (k_0), and between pH 9.0 and 11.0 by a hydroxide ion-catalyzed dehydration ($k_{\mathrm{OH^-}}$) of the neutral addition intermediate (Scheme 1):

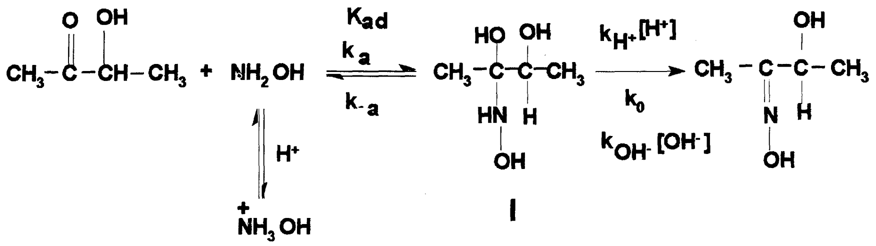
Equation 1[20)] is consistent with the experimental results:

$$k_{2\mathrm{obs}} = k_a \cdot k_{\mathrm{dap}}/(k_a + k_{\mathrm{dap}}) \tag{1}$$

Here, k_a can be obtained by Eq. 2 using the pseudo-first order experimental rate constant (k_{obs}) at pH < 1.5, where the attack of the amine on the carbonyl compound is rate-determining step. $[\mathrm{NH_2OH}]_1$ is the free base concentration.

$$k_a = k_{\mathrm{obs}}/[\mathrm{NH_2OH}]_1 \tag{2}$$

The value of k_{dap} is calculated by Eq. 3 using k_{obs} at pH > 1.5, where the dehydration of the addition intermediate is



Scheme 1.

rate-determining step.

$$k_{\text{dap}} = \{k_{\text{obs}}/[\text{NH}_2\text{OH}]_\text{l}\} \cdot fc = k_\text{d}K_{\text{ad}} \qquad (3)$$

$fc=1/(1+K_{\text{ad}}[\text{NH}_2\text{OH}]_\text{l})$. K_{ad}=equilibrium constant for the addition intermediate formation.

The rate constant (k_d) includes terms for the oxonium ion-catalyzed ($k_{\text{H}^+}=3.16\times10^5$ $\text{M}^{-2}\,\text{s}^{-1}$), the spontaneous ($k_0=3.37\times10^{-3}$ $\text{M}^{-1}\,\text{s}^{-1}$) and the hydroxide ion-catalyzed dehydration ($k_{\text{OH}^-}=1.83\times10^2$ M^{-2} s^{-1}). $kd=k_{\text{H}^+}[\text{H}^+]+k_0+k_{\text{OH}^-}[\text{OH}^-]$.

The theoretical solid line in Fig. 1 was calculated using Eq. 1 with the assistance of the experimentally-determined rate constants.

An analysis of the effect of calcium ions (0.1 M) in the total pH range studied showed that it increases the rate constant of the reaction at between pH 7.0 and 10.0. This region corresponds to the spontaneous dehydration as the rate-determining step of the reaction (see the broken line in Fig. 1).

The effect of the concentration of calcium ions on the rate constant was studied at pH 0.5, 7.5, and 8.5 (Table 1). At pH 0.5, where the rate-determining step is an uncatalyzed attack of the amine on the carbonyl compounds, no effect was observed. However, at pH 7.5 and 8.5 the increase in the calcium-ion concentration causes an increase in the rate constant, as shown in Fig. 2.

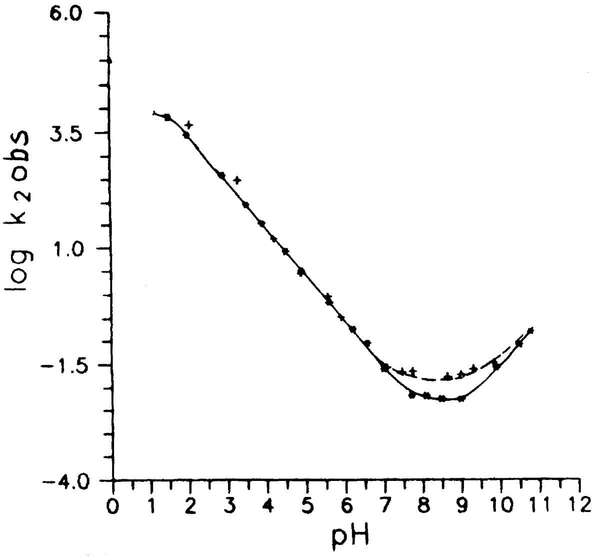


Fig. 1. pH dependence of the logarithms of second-order rate constants ($k_{2\text{obs}}$) for the reaction between acetoin (0.01 M) and hydroxylamine (0.1 M) in water at 25 °C, ionic strength 1.0 M (KCl). The broken line indicates the effect of calcium ions (0.1 M).

Table 1. Observed Second-Order Rate Constants for the Reaction between Acetoin and Hydroxylamine, in Presence of Calcium Ions at Different pH[a]

[CaCl$_2$]	k_2/M^{-1} s^{-1}		
M	pH=0.5	pH=7.5	pH=8.5
—	2.27×10^4	1.46×10^{-3}	0.46×10^{-3}
0.05	2.22×10^4	2.78×10^{-3}	1.48×10^{-3}
0.10	2.26×10^4	3.90×10^{-3}	1.99×10^{-3}
0.15	2.17×10^4	4.70×10^{-3}	2.47×10^{-3}
0.20	2.25×10^4	5.30×10^{-3}	2.81×10^{-3}

a) Water, 25.0 °C, μ=1.0 (KCl).

To interpret the mechanism of the calcium-ion effect, the equilibrium constant for the neutral addition intermediate formation, with and without the presence of calcium ions, was determined by the following two methods from the literature,[12,21] in order to obtain really trustworthy results. The values are $K_{\text{ad}}=1.22\pm0.06$ in the absence of calcium ion and $K_{\text{ad}}=0.91\pm0.09$ in presence of calcium ion (0.1 M), as given in Tables 2 and 3, respectively.

It is known that aliphatic α-hydroxy ketones exhibit in-

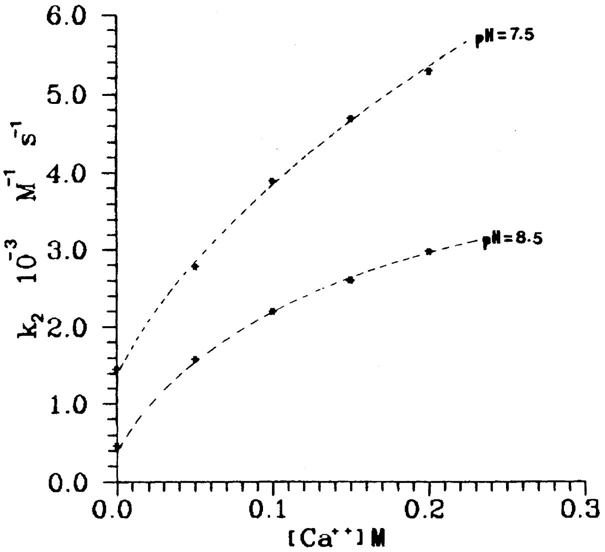


Fig. 2. Effect of the calcium ion concentration on the dehydration step at pH 7.5 and pH 8.5 for the reaction between acetoin (0.01 M) and hydroxylamine (0.1 M) in water at 25 °C, ionic strength 1.0 M (KCl).

Table 2. Equilibrium Constant Determinations for Addition Intermediate Formation from Hydroxylamine and Acetoin[a)]

$[NH_2OH]_t$	A_{b_x}	%C=O	%I	K_{ad}
0.05	0.980	0.945	0.055	1.16
0.10	0.918	0.885	0.115	1.29
0.15	0.879	0.847	0.152	1.20
0.20	0.833	0.803	0.196	1.22

a) Water, 25.0 °C, $\mu=1.0$ (KCl). pH=8.5 (morpholine buffer 0.01 M). Initial absorbance of ketone=1.037. $K_{ad}=1.22\pm0.06$.

Table 3. Equilibrium Constant Determinations for Addition Intermediate Formation from Hydroxylamine and Acetoin in Presence of 0.1 M $CaCl_2$[a)]

$[NH_2OH]_t$	A_{b_x}	%C=O	%I	K_{ad}
0.05	0.947	0.955	0.044	0.929
0.10	0.901	0.909	0.091	0.998
0.15	0.882	0.890	0.109	0.823
0.20	0.844	0.852	0.148	0.870
0.25	0.798	0.805	0.194	0.967

a) Water, 25.0 °C, $\mu=1.0$ (KCl). pH=8.5 (morpholine buffer 0.01 M). Initial absorbance of ketone=0.991. $K_{ad}=0.91\pm0.09$.

tramolecular hydrogen bonding.[22)] It was also demonstrated that α-hydroxy ketones form a complex with calcium ions, probably by the "α-chelation" mode.[23)] An analysis of acetoin in the presence of calcium ions by UV, IR, and ^{1}H NMR experiments has led to the consideration that there must exist complexation between these two chemical species at between pH 7.0 and 10.0. It is interesting to note that an analysis of the oxime in the presence of calcium ions shows that complexation also exists in the same pH range.

Metal ions often function as catalysts by polarizing reactive functional groups. In this case, the calcium ion may be able to polarize the carbonyl group, thereby facilitating a hydroxylamine attack; however, since there is no interaction between the acetoin and calcium ions in the pH region where the attack is the rate-determining step, no effect on the rate constant has been observed. (See Table 1).

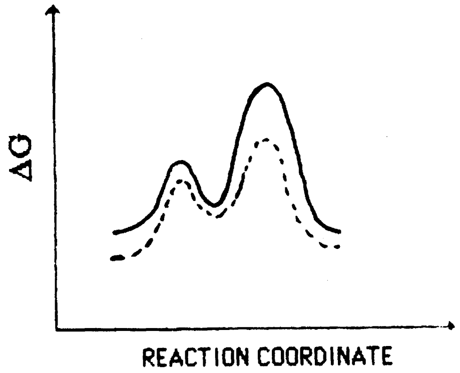


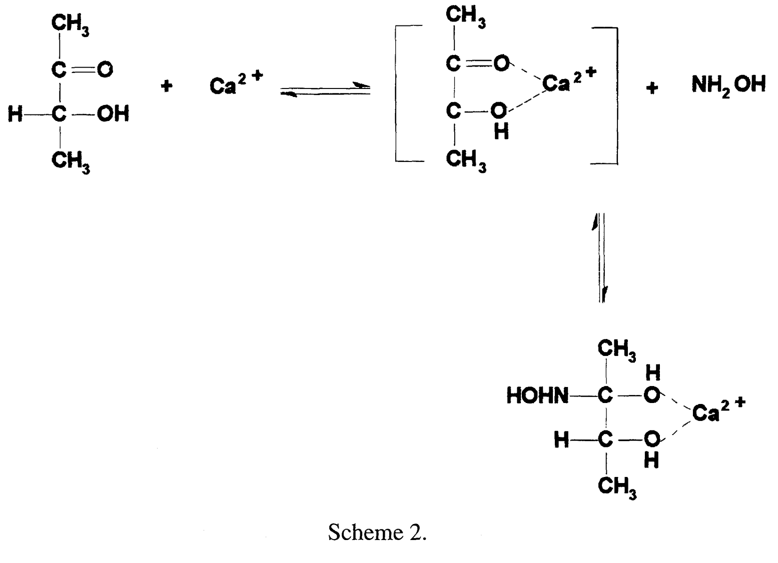
Fig. 3. Reaction coordinate diagram to illustrate the reaction between acetoin and hydroxylamine in presence (- - -) or in absence (——) of calcium ions.

In the Schiff-base formation from two molecules of *o*-aminobenzaldehyde with $[PtCl_4]^{2-}$, Sheeran and Mertes[24)] suggested that the metal ion was like a Lewis acid in stabilizing the tetrahedral addition intermediate.

According to Leussing,[9)] the primary kinetic role of the metal ions in this kind of reaction seems to be one of bringing the ligands together in a mixed complex without unduly restricting their ability to react further with each other.

The relationship between the observed rate constant and the calcium-ion concentration is not linear, as can be seen in Fig. 2. With an increase in the calcium-ion concentration, the rate constant initially increases, but then approaches a plateau. This kind of behavior indicates the existence of the formation of a complex between the neutral addition intermediate and the calcium ions, and that the rate-determining step is the breakdown of this complex.

To interpret the experimental facts observed in this reaction, where the presence of calcium ions decreases K_{ad} and increases the dehydration rate constant in the pH region of pH 7.0 to 10.0, it is necessary to assume that "α-chelation" between the calcium ions and acetoin further reduces the energy of the reagent, which in turn should decrease the energy



Scheme 2.

of the addition intermediate of the reaction (see Fig. 3 and Scheme 2).

The interaction between the calcium ions and the addition intermediate of the reaction between acetoin and hydroxylamine could be similar to that with sugars,[25)] i.e. between the calcium ions and two hydroxyl groups bonded to the carbon atoms of the addition intermediate. This kind of complexation should facilitate the departure of the hydroxide ion from the addition intermediate when the spontaneous dehydration is the rate-determining step, thus increasing the overall rate constant of the reaction (Fig. 3). The mechanism is similar to that demonstrated for boric acid in the dehydration step of similar reactions.[26)] These results lead us to consider the importance of calcium ions in the mechanism of the Maillard reactions.

Experimental

Kinetic Measurements: The reaction of acetoin with hydroxylamine in water at 25 °C (ionic strength 1.0 M (KCl)), was followed on a Varian DMS 80 spectrophotometer (equipped with a thermostated cell holder) by monitoring the formation of the oxime at 236 nm. The concentration of acetoin did not exceed 1.0×10^{-3} M. A sufficient excess of nitrogen base (0.05—0.20 M) was employed to ensure pseudo-first order kinetic behavior.

The determination of the rate constants was carried out using known procedures.[27)] The reactions were followed for three half-lives, and pseudo-first order rate constants (k_{obs}) were calculated by computer. Second-order constants ($k_{2\mathrm{obs}}$) were calculated from Eq. 1. The pK_a of NH_2OH used for a free-base calculation was 6.17.[21)]

The third-order rate constants ($k_{\mathrm{H^+}}$ and $k_{\mathrm{OH^-}}$) were obtained from the slopes of plots of the second-order constants against the concentration of the oxonium and hydroxide ion, respectively. The rate constant of a spontaneous reaction (k_0) and the uncatalyzed attack rate constant (k_a) were obtained directly from a plot of log $k_{2\mathrm{obs}}$ vs. pH.

Equilibrium Constants for the Addition Intermediate Formation: The equilibrium constant (K_{ad}) for the addition intermediate formation from hydroxylamine and acetoin was determined spectrophotometrically.[12,21)] This equilibrium constant was determined by measuring the decrease in absorbance at 272 nm of a solution of acetoin (0.01 M) in the presence of varying concentrations of hydroxylamine (0.05—0.25 M). The pH was maintained at 8.5 with 0.01 M of the morpholine buffer, and the ionic strength was adjusted to 1.0 with KCl. At this pH, since the dehydration of the addition intermediate is relatively slow, the observed absorbance could be extrapolated to zero time to determine the initial rapid decrease in the ketone absorbance (A_{b_x}) caused by the addition intermediate formation. The calculated values of the fraction of ketone converted to an addition intermediate (**I**) were based on an assumed absorbance of zero for the addition compound at 272 nm (A_{b_0}). Thus, the first method for calculating the equilibrium constant was, considering the measurements in triplicate for each concentration of hydroxylamine, from the equation $K_{\mathrm{ad}}=I/\{(1-I)\}$[hydroxylamine]. In the second method, K_{ad} was determined from the negative abscissa intercept of a plot of $1/\Delta A$ against 1/[amine], using Eq. 4, where ΔA is the change in the absorbance resulting from a conversion of the ketone to the addition intermediate:

$$1/\Delta A = 1/[\mathrm{C{=}O}]_t + 1/K_{\mathrm{ad}}[\mathrm{C{=}O}]_t \times 1/[\mathrm{amine}]. \qquad (4)$$

The obtained values are the average: $K_{\mathrm{ad}}=1.22\pm0.06$ in the absence of calcium ion and $K_{\mathrm{ad}}=0.91\pm0.09$ in presence of calcium ion (0.1 M).

Spectra: The ^{1}H NMR spectra were measured at 400 Hz in $CDCl_3$ on a Brucker AC 200F instrument with TMS as an internal standard. The IR spectra were recorded on a thin film using a Perkin–Elmer instrument (model IGPC FT-IR).

Acetoin–Ca^{2+} Complex Determination: UV spectroscopy shows that the maximum absorbance of the carbonyl group in 272 nm increases as a function of the calcium-ion concentration. In the IR spectra of a film of a mixture (1 : 1) of acetoin and $CaCl_2$, the $\nu_{\mathrm{C=O}}$ bond of acetoin shifts from 1716 cm^{-1} to 1712 cm^{-1} and the ν_{OH} bond from 3434 cm^{-1} to 3402 cm^{-1}. The ^{1}H NMR spectra ($ClCD_3$) of a mixture of acetoin and $CaCl_2$ in the same relation used in the kinetics experiments shows that the bond of the hydrogen-bonded on the 3-position shifts from 4.265 to 4.314 ppm. These experiments indicate the existence of a coordination of calcium ion with acetoin through the hydroxyl and carbonyl groups.

Oxime-Ca^{2+} Complex Determination: UV spectroscopy has shown that the maximum-absorbance value of the double bond, $>$C=N, at 224 nm decrease as a function of the calcium-ion concentration.

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