# Metal-ion Assisted Catalysis of Nucleophilic Attack. Part I. Zinc–anion Co-operation in Aldehyde Hydration

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The hydration of acetaldehyde has been studied as a 'typical' reaction involving nucleophilic attack upon the carbonyl group. This hydration is catalysed by zinc ions and the catalysis is enhanced by the presence of anions, acetate, and hydroxide in the systems studied. The co-operation between zinc and these ions in catalysis has been subjected to kinetic investigation and certain mechanistic deductions have been made. The relevance of this study to the mechanism of action of the hydrolytic enzyme carbonic anhydrase is discussed.

THE enzyme carbonic anhydrase is responsible for the catalysis of reactions involving the hydration of carbonyl centres. These include the hydration of $CO_2$ to hydrogen carbonate, hydration of aldehydes and hydrolysis of esters.[1,2] Carbonic anhydrase isozymes are small, consisting of a single polypeptide chain of molecular weight 30,000 and a firmly-bound zinc ion. This zinc ion, situated at the active site, is co-ordinated to three protein ligand groups; its fourth tetrahedral co-ordination site is open to a cavity in the enzyme, and reaction takes place when substrates enter this cavity.[3] There has been some controversy about the mechanism of this reaction, but the balance of recent evidence points to the involvement of zinc as a source of hydroxide ions; a water molecule in the fourth co-ordination site undergoes ionisation facilitated by co-ordination to a di-cation and the hydroxide thus produced attacks the carbonyl group, which is bound in a suitable adjacent position.[2,3]

Various simple models have been constructed in order to reproduce the behaviour of carbonic anhydrase.[4] Most of these have involved a free metal ion and carbonyl compound; the latter sometimes with a second ligating group to promote co-ordination to metal. However, the outstandingly high catalytic efficiency of carbonic anhydrase suggests that its operation calls *inter alia* for the involvement of more than one group in the enzyme. To pursue this, we have investigated the co-operative catalysis of a typical carbonyl hydration by zinc ions and other entities. The reaction chosen was the hydration of acetaldehyde.

Not many studies of co-operative catalytic effects in labile systems have been done but an indication of the effects to be expected has been obtained by Pocker and Meany,[5] who showed that acetaldehyde hydration was weakly catalysed by zinc ions in various buffers, but more strongly in imidazole buffers. Our attempts to extend this work with imidazole proved unsuccessful for two reasons: first, the permissible variation in imidazole and zinc concentration is quite small, because imidazole is not a strong ligand, with the result that zinc hydroxide precipitates at pH values sufficiently alkaline to deprotonate a substantial fraction of the imidazolium ion present, and second, interfering side effects in imidazole buffers were observed (see Experimental section). However, it was found that there is a moderately strong co-operative catalysis of acetaldehyde hydration by zinc and acetate ions. Acetate has a more suitable pH range for use with free zinc ions and was further investigated.

## EXPERIMENTAL

*Kinetic Apparatus.*—The first-order disappearance of acetaldehyde was followed utilising the carbonyl absorption at 276 nm ($\varepsilon$ 16 l $mol^{-1}$ $cm^{-1}$). The highest observed rate constants were around 0·1 $s^{-1}$, which is too fast for sampling or for repeated optical density measurements. An ap-

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[1] See, *e.g.*, J. T. Edsall, *Ann. New York Acad. Sci.*, 1968, 151; R. G. Khalifah, *J. Biol. Chem.*, 1971, **246**, 2561.

[2] R. H. Prince and P. R. Woolley, *Angew. Chem.*, in the press.

[3] J. Coleman, 'Metal Ions in Enzymatic Catalysis, in *Progr. Bio-organic Chem.*, eds. E. T. Kaiser and F. J. Kézdy, 1971, **1**, 159; K. Kannan, personal communication.

[4] A. E. Dennard and R. J. P. Williams, *J. Chem. Soc.* (*A*), 1966, 812; Y. Pocker and J. E. Meany, *J. Phys. Chem.*, 1968, 72, 655.

[5] Y. Pocker and J. E. Meany, *J. Amer. Chem. Soc.*, 1967, **87**, 1809.

paratus was built to plot u.v. transmission against time on a Servoscribe pen recorder. The monochromator and thermostatted cell housing from a Unicam SP 500 spectrophotometer were used; the SP 500 photocell box was replaced by an assembly comprising an IP28 photomultiplier fed by a stabilised 1 kV negative power supply. The output was taken from a 3·3 MΩ resistor in the photomultiplier's anode circuit and amplified by an EIL model 23A pH-meter connected as an infinite-impedance millivoltmeter. Output was taken from this to the pen recorder.

All experiments were conducted at $0{\cdot}0 \pm <0{\cdot}2$ °C, the cell mounting being cooled by water–glycol from a thermostatted bath. The cell temperature was checked regularly between runs. A slow stream of compressed air through the cell compartment prevented condensation.

The procedure for a kinetic run was as follows: the signal from the photomultiplier was cut off using the millivoltmeter's ' check ' position and the Servoscribe pen set to 0% using the zero adjustment. With a 1-cm quartz cell filled with 2·5 ml thermally equilibrated buffer in the light beam, the millivoltmeter was switched to ' read ' and the pen recorder's adjustable scale set to read 100% on the chart. A known volume, usually 5 μl, of acetaldehyde was then introduced rapidly from a chilled microsyringe; the solution was stirred and the chart paper set in motion. Readings could be taken from the chart within less than 10 s of starting to add the acetaldehyde, and these represented the percentage of light absorbed by the acetaldehyde alone. Thus it was possible to check that no process other than the hydration was occurring, since the transmission both at $t = 0$ and at $t = \infty$ should depend solely on the amount of acetaldehyde added.*

*Rate Data.*—Data points were read from the chart. For a first-order reaction, transmission ($T_t$) as a function of time ($t$) is given by

$$T_t = T_\infty \times 10^{-\mathrm{A}\exp(-k_{\mathrm{obs}}\,.\,t)}$$

where A is an arbitrary constant and $k_{\mathrm{obs}}$ the first-order rate constant. By taking logarithms twice, the expression can be made linear in $t$, thus:

$$\log_e [\log_{10} T_\infty - \log_{10} T_t] = -k_{\mathrm{obs}}\,.\,t + \log_e \mathrm{A}$$

$k_{\mathrm{obs}}$ And its standard deviation were found using linear least-squares formulae. Slow runs were treated by the analogous Guggenheim method. This saved time and minimised the possible error due to instrumental drift.

In processing the data, the rate plots were analysed using a programme on the Cambridge University Computer. Any first-order plot whose gradient had a standard deviation exceeding 1% was rejected. Most of the catalytic solutions were made up in 10-ml amounts, so that runs could be triplicated; the standard deviation of the mean of three acceptable runs was usually *ca.* 1%. More complex curve-fitting was carried out using a computer programme kindly made available by Dr. G. M. Sheldrick, of this department.

*Interpretation of* $k_{\mathrm{obs}}$.—We are dealing with reaction:

$$\mathrm{CH_3{\cdot}CHO} + \mathrm{H_2O} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \mathrm{CH_3{\cdot}CH(OH)_2}$$

All catalytic species are at constant concentration, and the water concentration varies negligibly. The rate is first-order in acetaldehyde with an observed rate constant equal to $(k_+ + k_-)$ where $k_-$ and $k_+$ are first-order or pseudo-first order rate constants. For the hydration we are interested in $k_+$ alone, but $k_{\mathrm{obs}}$ is easily converted to $k_+$ by multiplying $k_{\mathrm{obs}}$ by the hydration fraction, $f$, of acetaldehyde; it is easily shown that

$$f = \frac{[\mathrm{CH_3{\cdot}CH(OH)_2}]}{[\mathrm{CH_3{\cdot}CHO}] + [\mathrm{CH_3CH(OH)_2}]} = \frac{k_+}{k_+ + k_-}$$

At a fixed temperature and ionic strength $f$ will be constant, so all the rate constants quoted in this paper refer to $(k_+ + k_-)$ which is proportional to $k_+$; $k_+$ may be obtained by multiplying by the hydration fraction, *ca.* 0·78.

*Materials.*—Acetaldehyde was distilled and kept under nitrogen. All other materials were AnalaR grade.

Apart from the experiments mentioned in Table 1, all solutions were made up to ionic strength 1·4. The only anion added other than acetate was perchlorate; $HClO_4$ was used to make up the buffers and $NaClO_4$ as an inert salt. Zinc perchlorate solution was prepared from AnalaR zinc oxide and standard acid.

## RESULTS

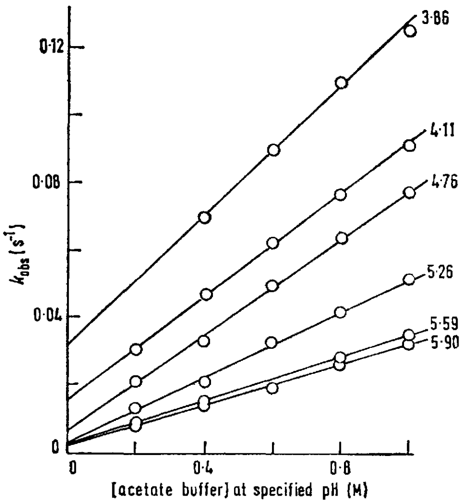

FIGURE 1 Hydration of acetaldehyde catalysed by acetate buffer at different pH values (0 °C, μ = 1·0)

(a) *Comparison with Earlier Work.*—To check the accuracy and reproducibility obtainable from the kinetic apparatus, the catalysis of acetaldehyde hydration by acetate buffers was examined. (*Note.* All experiments described in this paper were carried out at 0 °C.) The results are shown graphically in Figure 1 and the rate constants obtained are shown in Table 1. These figures are self-consistent, yet they differ considerably from those

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* Pocker and Meany [5] used this kind of reasoning to argue for the existence of a rapidly-formed carbinolamine intermediate when the catalyst is imidazole. The formation constant of this intermediate was reported to be about 0·14 l mol$^{-1}$. Our experiments with imidazole buffers suggest a stronger association, with formation constant nearer 6 l mol$^{-1}$. In addition we have observed another reaction at times much greater (20 min) than the typical half-life (20 s) of the imidazole-catalysed hydration. This was manifested by a slow *increase* in absorption at 276 nm. For these and other reasons the work with imidazole was not continued (see above).

obtained at the same temperature by other workers.[6a] The reaction medium used by them was 10% acetone in water with a much lower ionic strength, 0·10M. Accordingly the hydration was carried out at several ionic strengths, and a smooth curve with a slight negative gradient was obtained (Figure 2); this clearly does not extrapolate to the kind of value required by the results of reference 6*a*. The other factor which may be responsible for this discrepancy is the presence of 10% acetone in the reaction medium used by the earlier workers. As pointed out by Franks and others [7] many properties of water are profoundly affected by small concentrations of non-polar solutes, and 10% acetone might be enough to affect substantially the rate constant, particularly as the acetone was itself in ten-fold excess over the reactant acetaldehyde. This could not be checked with 10% acetone, as the acetone carbonyl u.v. absorption masks the absorption due to acetaldehyde, making the reaction impossible to follow spectrophotometrically. Kinetic runs were carried out in 0, ½, 1, and 1½% acetone, and the rates decreased significantly. With a typical acetate buffer the observed rate constants were 0·041, 0·046, 0·039, and 0·030 s$^{-1}$ respectively; plots were less accurate than usual owing to the approach towards the limit of sensitivity of the apparatus.

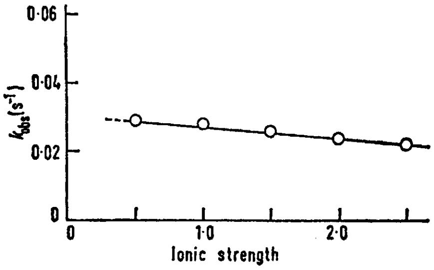

FIGURE 2 Hydration rate as a function of ionic strength; the scale is the same as that of Figure 1

TABLE 1

Catalytic constants (l mol$^{-1}$ sec$^{-1}$) for hydration of acetaldehyde in acetate buffers at 0 °C

| | Measured in $H_2O$, $\mu = 1{\cdot}0$ | Measured in 10% $Me_2CO$–$H_2O$ $\mu = 0{\cdot}1$ [6a] |
|---|---|---|
| $k_{H^+}$ | 225 ± 16 | 50 |
| $k_{HOAc}$ | 0·100 ± 0·007 | 0·053 |
| $k_{OAc^-}$ | 0·031 ± 0·005 | 0·0072 |

$k_{HOAc}$ and $k_{OAc}$ are consistent within experimental error with the composite terms obtained the Job's plots (Table 3 and Figures 5, 6) at zero zinc concentration.

(b) *Catalysis by Zinc Ions.*—This was examined in several acetate buffers. The observed rate constant plotted against [Zn$^{2+}$] gave a straight line in each case, and the gradient is the apparent catalytic constant $k_{Zn^{2+}}$. These are shown in Table 2 and Figure 3. It is quite clear from the data at pH 5 that the buffer somehow assists the zinc ions in catalysing the hydration. However, a plot of rate against [buffer] at pH 5 did not give a good straight line (Figure 4).

TABLE 2

Catalytic constants for Zn$^{2+}$ (0 °C, $\mu = 1{\cdot}4$)

| pH | [Acetate buffer] | $1000k_{Zn^{2+}}$ (l mol$^{-1}$ s$^{-1}$) [a] | $1000k_{Zn^{2+}}$ (corrected) (l mol$^{-1}$ s$^{-1}$) [b] |
|---|---|---|---|
| 4 | 0 | — | 11·5 ± 0·2 [c] |
| 5 | 0·01 | 13·5 ± 1·0 | 13·2 ± 1·0 |
| 6 | 0·05 | 19·0 ± 1·2 | 16·9 ± 1·5 |
| 6 | 0 | — | 17·8 ± 0·45 [c] |
| 5 | 1·0 | 96·8 ± 4·9 | — |

[a] Observed $k_{Zn^{2+}}$; the gradient of the plot of $k_{obs}$ against total [Zn$^{2+}$]. [b] Corrected, using equation (2), to allow for the co-operation between zinc and acetate; this number therefore represents catalysis by zinc ions as buffer concentration tends to zero. [c] Obtained from Job's plot parameters, extrapolating to zero acetate. The estimated contribution to these data by H$^+$, OH$^-$, and $H_2O$ is negligible. The independently-derived values for pH 6 are in good agreement.

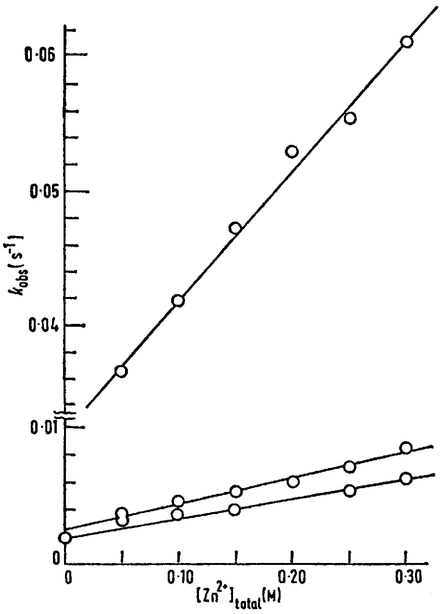

FIGURE 3 The catalytic effect of zinc ions (0 °C, $\mu = 1{\cdot}4$). Upper line, pH = 5·0 in 1M-acetate buffer; lower line, pH = 5·0 in 0·01M-acetate; middle line, pH = 6·0 in 0·05M-acetate

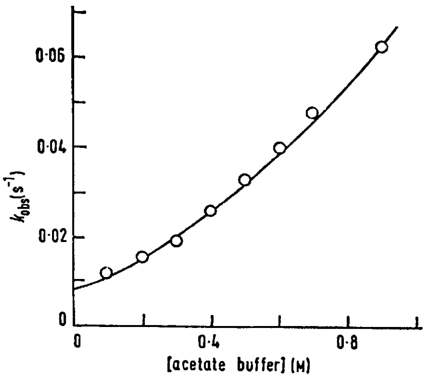

FIGURE 4 $k_{obs}$ Plotted against acetate buffer concentration in the presence of 0·25M-zinc ions; 0 °C, pH = 5·0, $\mu = 1{\cdot}4$. The curve represents the best-fit quadratic (see text)

To pursue this, Job's method of continuous variations was used. In this method the rate constant observed is plotted against a co-ordinate *x*, which varies from 0 to 1.

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[6] (*a*) R. P. Bell, *Adv. Phys. Org. Chem.*, 1966, **4**, 1, and references therein, particularly *Trans. Faraday Soc.*, 1950, **46**, 34; (*b*) R. P. Bell, M. H. Rand, and K. M. A. Wynne-Jones, *ibid.*, 1956, **52**, 1093.

[7] F. Franks, *Chem. and Ind.*, 1970, 1397; F. Franks and D. G. Ives, *Quart. Rev.*, 1966, **20**, 1.

To investigate the co-operativity between zinc and buffer, a range of solutions were made up with ionic strength 1·4, pH = 6, $[Zn^{2+}] = 0{\cdot}5x$, and $[\text{buffer}] = 0{\cdot}5(1 - x)$. Thus both $[Zn^{2+}]$ and [buffer] are linear functions of $x$, and since the reaction is first order in zinc, acetate ion, and acetic acid separately, any co-operativity or antico-operativity in the catalysis should be manifested as positive or negative deviation from a straight line in the plot of $k_{obs}$ against $x$.

The results of Job's plots carried out at pH 6 (at which pH the buffer exists *ca.* 95% as acetate ion) and at pH 4 (at which it is *ca.* 80% acetic acid) are shown in Figures 5 and 6. There is a fairly strong co-operativity at pH 6, showing that the reaction is accelerated by the joint action of zinc and acetate ions. None, however, is observed at pH 4. (It might be expected that some co-operativity would be found at pH 4, on account of the non-negligible fraction of ionised buffer. However, the rates are greater at pH 4, as the $H^+$-catalysed path is 100 times faster than at pH 6, and $CH_3CO_2H$ is a better catalyst than $CH_3CO_2^-$; as a result all $k_{obs}$ are considerably greater. Calculations based on results from the kinetic analyses below show that the effect of the much smaller co-operativity would be less than the increased error.)

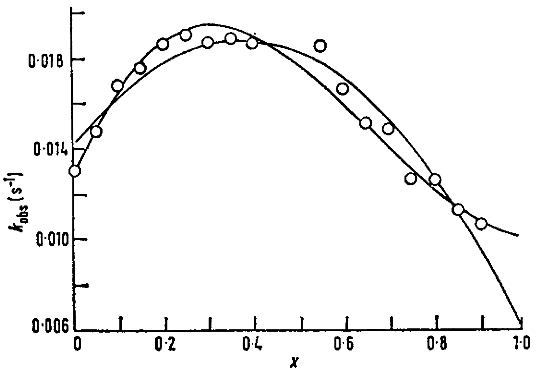

FIGURE 5 Job's plot showing co-operativity between zinc ions and acetate buffer at pH 6·0 (0 °C, $\mu$ = 1·4)

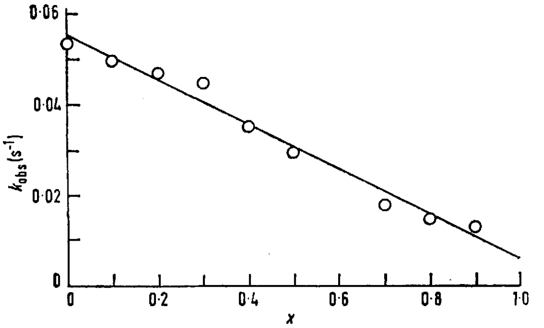

FIGURE 6 Job's plot showing lack of co-operativity between zinc ions and acetate buffer at pH 4·0 (0 °C, $\mu$ = 1·4)

*Kinetic Analysis.*—The co-operative catalysis by zinc ions and acetate ions (Figure 5) shows a maximum deviation from linearity, *i.e.* from the linear baseline, in the region $x = 0{\cdot}3$ to 0·4. Analysis of the appropriate kinetic equations predicts a maximum at $x = 0{\cdot}5$ if the co-operative phenomenon is due to equal numbers of zinc and acetate ions, and at $x = 0{\cdot}33$ if the zinc : acetate ratio required is 1 : 2. Thus the ratio of $Zn^{2+}$ to buffer (taken to be $CH_3CO_2^-$; see below) in the transition state is apparently 1 : 2 and since the orders are unity in zinc and in acetaldehyde, the transition complex should contain 1 acetaldehyde molecule, 1 zinc ion, and 2 acetate ions. However, computer curve-fitting showed that this was not conclusively so. It was carried out as follows.

The general kinetic equation was taken to be equation (1). This reduces at constant pH to (2), and on the assumption that co-operative hydration is exclusively first or second order in acetate this is reduced to (3) or (4) respectively.

$$k_{obs} = k_{H_2O} + k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{HOAc}[HOAc] + k_{OAc^-}[OAc^-] + k_{Zn^{2+}}[Zn^{2+}] + k_2[Zn^{2+}][OAc^-] + k_3[Zn^{2+}][OAc^-]^2 \quad (1)$$

$$k_{obs} = A + Bx + \tfrac{20}{19}k_2x(1 - x)/4 + (\tfrac{20}{19})^2k_3x(1 - x)^2/8 \quad (2)$$

$$k_{obs} = A + Bx + \tfrac{20}{19}k_2x(1 - x)/4 \quad (3)$$

$$k_{obs} = A + Bx + (\tfrac{20}{19})^2k_3x(1 - x)^2/8 \quad (4)$$

Equations (2)—(4) are derived making the substitutions $[Zn^{2+}] = 0{\cdot}5x\text{M}$ and $[\text{buffer}] = 0{\cdot}5(1 - x)\text{M}$. The factor $(\tfrac{20}{19})$ arises from the fact that 95% of the buffer exists as $CH_3{\cdot}CO_2^-$.

The data of the Job's plots at pH values of 4 and 6 were fitted in turn to equations (3) and (4); A, B, $k_2$, and $k_3$ were refined simultaneously using a least-squares method in which all points were weighted equally. The parameters and $R$ values * corresponding to best fit are shown in Table 3. Two points emerge: first, that a second-order expression in acetate fits the data better than first order, and second, that the inclusion of a first-order and a second-order term fits them better still. However, the $F$ test shows that while the fit with equation (2) is significantly better, *i.e.* to at least 95% confidence, than that with equation (3), the differences between the fits of equations (2) and (4) and equations (3) and (4) are not significant to that level. It therefore seems likely, but not certain, that the co-operative path involves optionally either one or two acetate ions. We therefore adopt equation (2) as an empirical representation of the data.† The existence of a

TABLE 3

Parameters for Job's plot at pH 6—all × $10^3$

| Equation fitted | A ($s^{-1}$) | B ($l\ mol^{-1}\ s^{-1}$) | $k_2$ ($l^2\ mol^{-2}\ s^{-1}$) | $k_3$ ($l^3\ mol^{-3}\ s^{-1}$) | R (%) |
|---|---|---|---|---|---|
| (2) | 13·1<br>±0·4 | −4·59<br>±0·99 | 50·8<br>±21·1 | 300 ± 63 | 2·7 |
| (3) | 12·8<br>±0·4 | −2·46<br>±0·51 | | 442 ± 27 | 3·2 |
| (4) | 14·1<br>±0·5 | −8·76<br>±0·72 | 144 ± 13 | | 4·5 |

Note: A and B define the baseline; these terms are composite and can therefore be negative.

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* $R$ value: defined as $\surd\{(\text{sum of squared errors})/(\text{sum of squared observed values})\}$, summed for all data points, where error = {(observed result) − (result calculated from best-fit parameters)}.

† It should be noted that the use of equation (2) leads to considerable uncertainty in the values of $k_2$ and $k_3$, as Table 3 shows; however this is inevitable as the data of Figure 5 are not very sensitive to order in acetate.

path second order in acetate seems unquestionable; the existence of a parallel first-order pathway is less certain.

As mentioned above, the Job's plot at pH 4 showed no significant co-operation between zinc ions and buffer. This plot was subjected to the same curve-fitting procedure, and the $k_2$ and $k_3$ were found to be insignificantly different from zero.

As a check on these parameters, the curve shown in Figure 4 was fitted to equation (1), simplified so as to bring out the acetate dependence, as in equation (5):

$$k_{\mathrm{obs}} = \mathrm{A} + \mathrm{B}[\mathrm{OAc}^-] + \mathrm{C}[\mathrm{OAc}^-]^2 \qquad (5)$$

C in this equation corresponds to $k_3$. Its value was found to be (218 ± 43) $\mathrm{l^2\,mol^{-2}\,s^{-1}}$, which corresponds roughly with the Job's plot value of (300 ± 63) $\mathrm{l^2\,mol^{-2}\,s^{-1}}$. However, it is hard to separate B and C in equation (5), which represents a fairly steep line with only moderate curvature. The $\mathrm{p}K_\mathrm{a}$ of acetic acid at 0 °C and ionic strength 1·4 was taken as 4·6 (based on data in ref. 8*a*).

## DISCUSSION

It emerges from the kinetic analysis that the co-operative catalysis of acetaldehyde hydration by zinc and acetate involves a transition complex of formula $[\mathrm{Zn{\cdot}CH_3CHO{\cdot}(CH_3CO_2^-)}_n]$, where $n$ can be 1 or 2. In addition the complex will contain a number of water molecules. The acetate must be in its anionic form.

In addition, the catalytic constant for zinc alone increases between pH values of 4 and 6. Thus it seems that zinc and hydroxide ions exhibit a co-operative effect similar to that shown by zinc and acetate ions. However, the kinetics of the process do not give any direct insight into the nature of the co-operation.

There are basically two ways in which acetaldehyde hydration can be catalysed (Figure 7): either by facilitating protonation of the carbonyl oxygen (as done by a general protic, or Brønsted, acid), or by facilitating nucleophilic attack at the carbonyl carbon. The latter may be effected either by activating a water molecule (general base catalysis or transfer of metal-bound hydroxide) or by polarising the carbonyl group; such polarisation may be induced by co-ordination through oxygen to a Lewis acid, including $\mathrm{H^+}$. If this Lewis acid is a metal ion, the complex formed must be labile enough to release the hydrated species after nucleophilic addition has occurred.

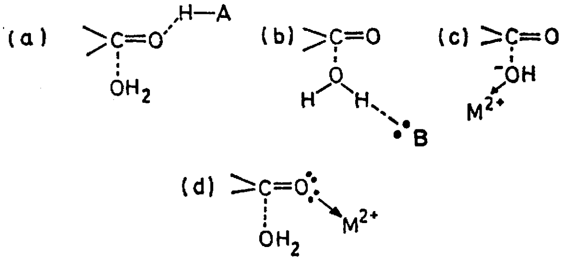

FIGURE 7 Hydration of acetaldehyde. Catalysis (a) by a general protic acid; (b) by a general base; (c) by transfer of metal-bound hydroxide; (d) by polarisation of the C=O bond by a Lewis acid

Now the aquated zinc ion could act as a catalyst in any of these ways; as a general acid through one of its 12 ionisable protons, as a hydroxide carrier and thus as a specific base in the form $[\mathrm{(H_2O)_5ZnOH}]^+$, or as a labile Lewis acid capable of co-ordinating acetaldehyde. These, shown in Figures 8 (a)—(d), will be considered in turn.

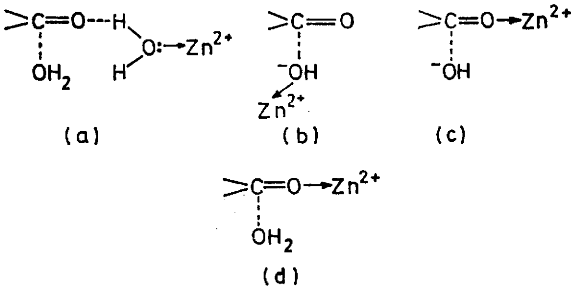

FIGURE 8 (a)—(d) Hydroxide-dependent and -independent paths involving $\mathrm{Zn^{2+}}$. See text

If $\mathrm{Zn(OH_2)_6}^{2+}$ acts as a general Brønsted acid, assisting protonation on the carbonyl oxygen, then one should be able to obtain a crude estimate of Brønsted's α by comparing its catalytic constant with that of acetic acid. Such a comparison, assuming $\mathrm{p}K_\mathrm{a}$ of $\mathrm{Zn(OH_2)_6}^{2+}$ to be 9·2 and that of acetic acid 4·6,[8] yields an α of 0·18; comparison of this with the value 0·54 derived by Bell *et al.*[6b] does not support the general acid path. In addition, this explanation fails to account for the co-operation between zinc ions and acetate. While it is reasonable to suppose that acetaldehyde should be able to undergo simultaneous general-base *and* Brønsted acid catalysis, there is no reason why co-operation of that kind should be restricted to zinc ions as acid; one would equally expect co-operation between acetate ions and acetic acid. Yet this has not been observed.

The second possibility is that the zinc might be acting as a hydroxide carrier, as it is supposed to do in carbonic anhydrase. This is consistent with the observed pH-dependence of $k_{\mathrm{Zn^{2+}}}$. In order for co-operation between zinc and acetate to be observed a transition state such as in Figure 9 could be imagined, in which the acetate assists in deprotonating the zinc. Requirement for such assistance is reasonable, as the deprotonation rate of a given water molecule in $\mathrm{Zn(OH_2)_6}^{2+}$ is quite slow (assuming a diffusion-controlled protonation rate of $10^{10}\ \mathrm{l\,mol^{-1}\,s^{-1}}$, and a p$K$ for two protons of 10, then the dissociation rate is only 1 $\mathrm{s^{-1}}$), but there seems no reason why the reaction rate should not be still further enhanced by simultaneous *O*-protonation by a general acid. Indeed, this might be thought to account for the second-order term in the rate equation, which at a given pH can only be described as $[\mathrm{buffer}]^2$ and therefore might be $[\mathrm{CH_3CO_2H}][\mathrm{CH_3CO_2^-}]$ rather than $[\mathrm{CH_3CO_2^-}]^2$. However, at pH 6 the concentration of acetic acid is

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[8] (*a*) L. G. Sillén and A. E. Martell, ' Stability Constants,' *Chem. Soc. Special Publ.*, No. 17, 1964; (*b*) *ibid.*, Suppl. No. 1, 1969.

very small, so that the rate constant $k_3$ would have to be very large; this would result in a very much *stronger* co-operativity at pH values of 5 and 4, whereas in fact a much weaker one is observed at these pH values. A further argument against the acetate-assisted transfer of zinc-bound $\mathrm{OH^-}$ is that there is no reason to believe a water molecule H-bonded to acetate *and* co-ordinated to $\mathrm{Zn^{2+}}$ to be a better nucleophile than a free water molecule.

The other suggestion is that the zinc ion acts as a Lewis acid catalyst, while the acetate acts as a general base (Figure 10). This is still not an entirely satisfactory explanation, as there is no obvious function for the second acetate ion. Nonetheless, it does not suffer from the weaknesses of the other paths suggested. By analogy, zinc and hydroxide should co-operate by the direct attack of hydroxide on the polarised, co-ordinated carbonyl group.

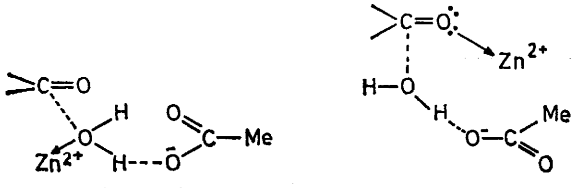

FIGURE 9 FIGURE 10

FIGURE 9 and 10 Possible co-operative paths involving $\mathrm{Zn^{2+}}$ and $\mathrm{CH_3CO_2^-}$. See text

It is possible to rationalise the pH-dependent rate constants for catalysis by zinc without acetate. Suggested paths for this are shown in Figure 8. Paths (b) and (c) require a kinetic term proportional to $[\mathrm{Zn^{2+}}][\mathrm{OH^-}]$, whereas in fact $k_{\mathrm{Zn^{2+}}}$, while increasing with pH, is not directly proportional to $[\mathrm{OH^-}]$. Nonetheless it may be separated into two terms, one constant and one hydroxide-dependent, giving for best fit the very approximate parameters: $k_{\mathrm{Zn^{2+}}} = (0{\cdot}0116 + 7 \times 10^5\,[\mathrm{OH^-}])$ l mol$^{-1}$ s$^{-1}$. One can interpret the constant term as paths (a) or (d); (d) is the same as (c) except that the nucleophile is $\mathrm{H_2O}$ rather than $\mathrm{OH^-}$.

The first path, Figure 8(a), is classic general acid catalysis and is implausible on the grounds discussed above.

The second path, Figure 8(b), is a second-order reaction if the zinc is already deprotonated. Thus the contribution of the path to $k_{\mathrm{obs}}$ is given by $k'[\mathrm{ZnOH^+}]$. But $[\mathrm{ZnOH^+}] = \dfrac{K_a[\mathrm{Zn^{2+}}]}{[\mathrm{H^+}]} = \dfrac{K_a[\mathrm{Zn^{2+}}][\mathrm{OH^-}]}{K_w}$, where $K_a$ is the ionisation constant of aquated zinc ions and $K_w$ the ionic product of water. Taking these to be $10^{-9}$ mol l$^{-1}$ and $10^{-14}$ mol$^2$ l$^{-2}$ respectively,* it follows that $k' = 7 \times 10^5 \times K_w/K_a = 7$ l mol$^{-1}$ s$^{-1}$. This is the bimolecular rate constant for the attack of zinc-co-ordinated hydroxide on free acetaldehyde, and the value is reasonable [*cf.* the same figures with $\mathrm{H_2O}$ as nucleophile ($1{\cdot}3 \times 10^{-5}$ l mol$^{-1}$ s$^{-1}$) and free $\mathrm{OH^-}$ (4000 l mol$^{-1}$ s$^{-1}$)[6a]].

On the basis of the third path, Figures 8(c) and (d), let a zinc–acetaldehyde complex be formed with stability constant $K_c$. Then considering first Figure 8(c), the rate term for the path would be $k''[\mathrm{Zn{\cdot}CH_3CHO}][\mathrm{OH^-}]$, which equals $k''[\mathrm{Zn^{2+}}][\mathrm{CH_3CHO}]K_c[\mathrm{OH^-}]$ as long as the complex is weak. Now, 1M-zinc perchlorate has no effect on the u.v. spectrum of acetaldehyde solution. Let us therefore suppose that the upper limit to the fraction of acetaldehyde co-ordinated to zinc is *ca.* 10%. The upper limit of the stability constant is then 0·1 l mol$^{-1}$. Since $k''K_c = 7 \times 10^5$ l mol$^{-1}$ s$^{-2}$, and $K_c \leqslant 0{\cdot}1$ l mol$^{-1}$, it follows that $k'' \geqslant 7 \times 10^6$ l mol$^{-1}$ s$^{-1}$, which is a good deal in excess of the corresponding rate constant for the attack of hydroxide ion upon free acetaldehyde [4000 l mol$^{-1}$ s$^{-1}$ (ref. 6*a*)]. Analogous treatment of the fourth, hydroxide-independent path gives a rate term of $k'''[\mathrm{Zn{\cdot}CH_3CHO}][\mathrm{H_2O}]$, with $k''' = 2 \times 10^{-3}$ l mol$^{-1}$ s$^{-1}$; again, this considerably exceeds the rate of attack of water on free acetaldehyde.

One possibility, not yet discussed, is that both the anion and the substrate are co-ordinated to zinc. This would result in a path for acetate as shown in Figure 11(a) and for hydroxide as Figures 11(b) or (c); in the hydroxide path the zinc is exercising simultaneous acidic and basic functions. With the evidence presently available there is no way of ruling out such a mechanism for the hydroxide path. However, the bound-acetate path seems unlikely for the following reason. The stability constant of the equilibrium $\mathrm{Zn(OH_2)_6^{2+}} + \mathrm{OAc^-} \rightleftharpoons [(\mathrm{H_2O})_5\mathrm{ZnO_2C{\cdot}CH_3}]^+$ at 0 °C and ionic strength 1·4M is not available in the literature, but extrapolation from the data given in reference 8*a* suggests that complex formation should only become significant at acetate concentrations rather higher than those employed here. $k_{\mathrm{Zn^{2+}}}$ Was measured at a very high concentration, 1M, at pH 5 (Table 2), and, although the co-operative effect is manifested, the observed $k_{\mathrm{Zn^{2+}}}$ (0·097 l mol$^{-1}$ s$^{-1}$) is a good deal smaller than that which the data from Table 3 would predict, *viz.* 0·186 l mol$^{-1}$ s$^{-1}$. (In making this prediction the change in buffer composition with pH was allowed for.) In addition, free acetate is not a very strong base, and co-ordinated acetate would probably be even less effective.

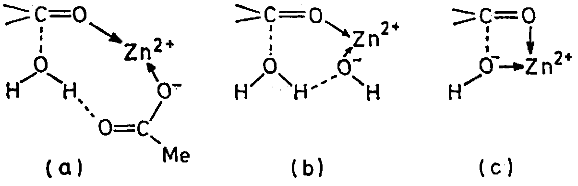

FIGURE 11 Simultaneous co-ordination of substrate and (a) acetate, (b) and (c) hydroxide; see text

It thus seems more likely that the co-operation between zinc ions and anions in the catalysis involves rate-limiting nucleophilic attack of hydroxide (either $\mathrm{OH^-}$ or $\mathrm{H_2O}$ + general base) upon the zinc-bound

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* Taking the observed p$K_a$ of $\mathrm{Zn(OH_2)_6^{2+}}$ to be 9·2, the p$K_a$ for a particular proton is (9·2 + log 12) and for a particular water molecule (9·2 + log 6).

carbonyl group. The hydroxide could be free or co-ordinated to the metal in an adjacent site. This bears an obvious relation to the mechanism of action of carbonic anhydrase since, as pointed out above, there is some evidence that for carbon dioxide (2) this enzyme operates through a mechanism involving co-ordinated nucleophile and unco-ordinated substrate; it would raise interesting problems if it could be shown that the simpler system utilises a different mechanism. The metal-ion catalysis of nucleophilic attack has been shown in some systems to proceed through separate paths, classified as ' free substrate, bound nucleophile ' and ' free nucleophile, bound substrate ',[9] and it could even be that the existence of such parallel mechanism is a general feature of this kind of catalysis. More detailed investigation of this reaction is in progress.

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