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Coordination Catalysis: Tautomeric *vs.* Carbanion Mechanisms in the Racemisation of L-Alanine Induced by Pyruvate and Zn^{2+} Ions

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The modification of ligand reactivity which occurs when aminocarboxylate ligands are coordinated to a wide variety of transition metal ions is now well understood. The increased reactivity which occurs even at sites other than donor atoms is clearly demonstrated by a variety of base catalysed reactions, including racemisation of optically active ligands, Knoevenagel type condensations with aldehydes and ^1H–^2H exchange, all at the 2-carbon methylene or methine group in coordinated 2-amino acids, and the general pattern of structure–activity relationships has recently been reviewed [1]. Using diamagnetic complexes of cobalt(III) it has proved possible to measure the rates of racemisation and isotope exchange concurrently, and thus to demonstrate that the reactions occur via a simple carbanion intermediate [2], Fig. 1. The same intermediate can also be invoked to explain the condensation reactions, but it is possible that transient imine formation may also be significant in the latter case [3].

More recently, however, it has been shown that the racemisation of L-alanine in the presence of Cu^{2+} ions occurs largely via a less direct mechanism [4], Fig. 2, and that although the simple carbanion intermediate can be formed, that route normally plays a relatively minor role in both racemisation and isotope exchange. The proposed major mechanism involves the copper catalysed oxidation of L-alanine to yield pyruvate, followed by condensation of pyruvate and L-alanine to yield the copper complex of the Schiff base {N-(1-carboxylatoethylidene-L-alanine)}. At room temperature the base catalysed reaction of the alanine moiety then occurs via carbanion formation in the coordinated Schiff base, but at elevated temperatures an alternative mechanism involving transamination also becomes important. These two competing routes may be distinguished through the pattern of deuterium incorporation which they generate if the reaction is carried out in alkaline ^2H_2O since the carbanion intermediate gives alanine labelled solely at the 2-carbon, but transamination leads to the replacement of hydrogen attached to both the 2 and 3-carbon atoms (Fig. 2).

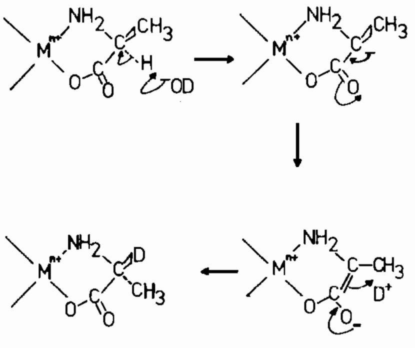

Fig. 1. The simple carbanion route for deuterium incorporation and racemisation.

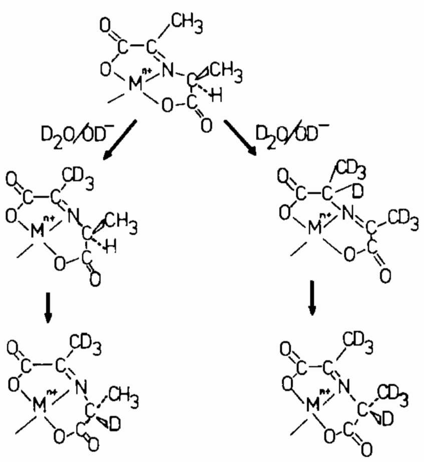

Fig. 2. Alternative pathways for activation via the coordinated Schiff base. Tautomerism leads to the 3C–D substituted product.

The kinetics of the pyruvate mediated reaction have been further investigated [5] and it was noted that zinc ions could also promote this reaction, though with slightly less effect than copper. However, the reaction was carried out at 60 °C and no comment was made as to the relative importance of the two mechanisms. We have therefore investigated this zinc induced reaction further in order to resolve this question and to derive further information about the relative efficiency of metal ions in promoting such reactions.

The direct reaction of L-alanine coordinated to Zn^{2+} in alkaline solution was examined first. Typically, using an excess (4–8 fold) of L-alanine to prevent precipitation of zinc hydroxide, the activating effect of the zinc ion was tested by attempting to

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observe either racemisation or ^2H incorporation. The study could only be performed over a limited pH range, pH $\leqslant$ 10 approximately, since higher concentrations of OH^- resulted in the precipitation of zinc hydroxide, even in the presence of large (8X) excess of ligand. No racemisation or deuterium incorporation was observed under any of the conditions used, that is with temperatures up to 80 °C for periods of days or even weeks. Whilst this observation does not entirely preclude some zinc induced racemisation over longer periods, it clearly demonstrates the almost negligible activating effect for the metal ion in simple combination with alanine. Zinc(II) is therefore considerably less effective than copper(II) in this role, since as described previously [4], using milder conditions (room temperature, pH < 10) than those used in this study, Cu^{2+} ions were still able to promote some, albeit limited, reaction even when steps were taken to suppress pyruvate formation and hence force reaction via the simple carbanion route.

The effect of pyruvate on the reactivity of the zinc–alanine system was examined next. At room temperature the addition of pyruvate first resulted in the slow formation of the Schiff base. This was clearly demonstrated by changes in the p.m.r. spectrum. Adding pyruvate resulted in the appearance of four new signals which grew in intensity as the proportion of pyruvate:alanine increased: 2 singlets (δ = 8.23, 7.4) and a new doublet, quartet pattern (δ = 8.33, 5.45) representing alanine condensed with pyruvate in the new Schiffs base. Likewise the original doublet, quartet pattern (δ = 8.32, 6.25) of the alanine reduced so that the overall intensity of the signals due to alanine was approximately constant. On standing this spectrum slowly changed with the disappearance of the pyruvate methyl signals only, over a period of several hours. Prolonged standing over a period of days or weeks resulted in no further changes which could be ascribed to any activation of the alanine. However, the spectrum was slowly degraded, probably as a result of the formation of pyruvate dimer and the observed precipitation of zinc hydroxide.

On heating a freshly prepared solution of Zn^{2+}: L-alanine:pyruvate in various proportion from 1:4:0.1 to 1:8:8 and over a pH range of 7–10 a different pattern of reactivity was observed. Initially, changes due to Schiff base formation and pyruvate methyl exchange could be observed, but more rapidly than before, but after 24 hours at 65 °C a significant reduction in both the alanine methine and methyl signals was observed. This reduction was greatest at the highest ratio of pyruvate: L-alanine and highest pH (pH = 10 approximately) and clearly implies reaction by a tautomeric route, Fig. 2. Unfortunately however such solutious slowly decompose under these conditions and no accurate measurement of kinetic constants was possible, though in agreement with an earlier report [5] it was observed that Zn^{2+} was less effective than Cu^{2+} in this reaction.

Clearly, the difference between the metals is subtle, but obviously factors such as relative stability constants and ligand exchange rates must play an important part in deciding the order of activating effect amongst the various divalent metal ions. It is interesting that the Zn^{2+} failed to promote any exchange in the simple Zn^{2+}: L-alanine system since similar observations have been reported with EDTA as ligand [6], where both Cu^{2+} and Ni^{2+} were able to promote exchange, but Zn^{2+} was not, and of the three the stability constant for the $[Zn^{2+}\ EDTA]^{2-}$ complex is least [7].

The addition of pyruvate to the Zn^{2+}: L-alanine system results in the formation of the coordinated Schiff base. The metal ion has a substantial structure promoting effect [8] since the formation constant for the pyruvate–alanine imine is ≅2 whilst for the 1:1:1 complex it is ≅10 [7]. However, it appears that zinc is still not able to promote 2C–H bond dissociation directly, at least in this system. If the pyruvate is replaced by other carbonyl fragments, notably pyridoxal, then the direct replacement of 2C–H via the carbanion form of the Schiff base can be observed [9]. Interestingly, though, heating the pyridoxylene system also leads to reaction via the tautomeric route. It would seem, therefore, that whereas with pyruvate and alanine the role of the Zn^{2+} ion is largely that of increasing the stability of the imine, unlike copper there appears to be little direct zinc induced activation. Unfortunately definite comparative data on complex stability and ligand exchange rates are lacking for this ligand, but certainly it must be expected that the zinc complex will be less stable than the copper analogue. One final difference may be the relative proportion of carbinolamine and imine in the coordinated ligand since the carbinolamine will be less effective and it has been suggested that the choice of metal ion may affect the ratio of these two forms [8]. What is certain though is that despite the large amount of information concerning Schiff bases the fine detail of their reactivity is still not entirely certain.

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