β-Proton Exchange in α-Amino- and α-Oxo-Acids: A New Metal Chelate-catalysed Reaction

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SCHIFFS bases formed with pyridoxal are intermediates[1,2] for many reactions of α-amino-acids, such as transamination, racemization, decarboxylation, and elimination of negative groups from the β-position of the amino-acid. The strong catalytic effects of metal ions on these reactions are ascribed to strong co-ordination of the Schiffs base nitrogen which results in conversion of the imine group to a negative donor group by dissociation of a proton from the α-position of the amino-acid (or alternatively by decarboxylation or by fission of the α–β carbon–carbon bond). Neutralization of the negative charge can occur by addition of a proton to the α-carbon of the amino-acid (racemization and α-proton exchange), addition of a proton to the α-carbon of pyridoxal (transamination), or by elimination of an electronegative group from the β-position of the amino-acid. So far, however, no

mechanism has been suggested for the exchange of the β-protons of the amino-acid. Here is presented the first kinetic evidence for vitamin B_6-catalysed β-proton exchange in the presence and absence of metal ions, as well as a mechanism for metal-catalysed and metal-free exchange reactions.

When valine (0·0050 mole), aluminium ion (0·00030 mole) (as aluminium sulphate), and pyridoxal hydrochloride (0·0010 mole) are dissolved in D_2O (10 ml.) and brought to pD 5·9 with sodium deuterioxide solution, no immediate reaction is detected by n.m.r. other than the formation of pyridoxylidene valine and its aluminium chelates. Since the majority of the material present is free valine, the prominent peaks of the spectrum are: a doublet at −4·09 p.p.m., the α-proton; a multiplet at −2·70 p.p.m., the β-proton; and four lines of nearly equal intensity with a centre at −1·50 p.p.m., the methyl resonances. All shifts are referred to an internal capillary or tetramethylsilane. When this solution is heated on a steam-bath for four days, the four-line methyl pattern gradually disappears and two quite broad singlets appear between each pair of doublet components separated by 0·05 p.p.m. and shifted upfield by *ca.* 0·01 p.p.m. An upfield shift is to be expected for deuterium substitution,[3] and the separation of the two broad singlets is identical to that observed for the magnetic non-equivalence of the normal valine methyl signals; therefore the new lines are due to β-deuteriated valine. Furthermore, when water is added and the reaction allowed to proceed, the normal four-line methyl signal returns. Integration of the spectra confirms this interpretation.

Similarly, when α-aminobutyric acid (0·0050 mole), pyridoxal (0·00050 mole), and aluminium ion (0·00020 mole) are dissolved in D_2O with the pD adjusted to 5·0, a triplet is initially observed at −1·47 p.p.m. (the acid methyl resonance). After 8 hr. on a steam-bath, the triplet is replaced by a fairly broad singlet and the α- and β-resonances vanish. During the exchange, no doublet is observed in the methyl region, which indicates that very little monodeuteriated amino-acid is formed. When H_2O is the solvent, the triplet is not altered and the α- and β-protons remain. A similar solution without aluminium in D_2O but with disodium EDTA (10^{-5} mole) exchanges negligibly in 16 hr. thus demonstrating the importance of the metal ion in this reaction. Copper(II) and zinc(II) also catalyse the β-exchange of α-aminobutyric acid.

To investigate the mechanism of this reaction, ketimine Schiffs bases formed from reactions between the α-oxo-acid and pyridoxamine and their metal chelates were examined. Pyruvate and pyridoxamine form a Schiffs base in which β-protons of the acid are exchanged with a half-life of about 45 min. at pD 7·2 and 0·10M ionic strength. At lower pD the reaction proceeds more slowly, probably partly because the Schiffs base is less stable in acid solution.[4] When α-oxobutyric acid is used, the exchange can be observed not only by the decrease in β-integral but also by the appearance of a doublet followed by a singlet in the γ-methyl region. The half-life in this case is *ca.* 2 hr. at pD 7·6. If the oxo-acids alone are dissolved in D_2O the exchange of β-protons proceeds quite slowly below pD 9·5. Pyruvate is reported to enolize with a rate constant of the order of 10^{-5} min.$^{-1}$ in neutral solution.†

When sufficient aluminium ion is added to form the 2 : 1 complex with the ketimine Schiffs base and the pD is lowered, the integral of the β-resonance is greatly reduced within a few min. which indicates a rapid exchange of the β-protons in the Schiffs base complex. The aluminium, however, also forms a strong complex with the oxo-acid and the spectrum is complex.

If the same experiment is performed with zinc(II) an initial rapid decrease of most of the β-proton signal is observed followed by a slow decrease of the remainder. In the region of pD 8 where the Schiffs base occurs, inhibition of this process is caused, probably by complex formation between zinc and pyridoxamine, and there is a corresponding inhibition of exchange. A similar phenomenon has been reported for the metal-catalysed transamination of oxo-acids.[5] If the pD of the zinc complex solution is maintained at $\geqslant 8$ exchange occurs in one rapid phase.

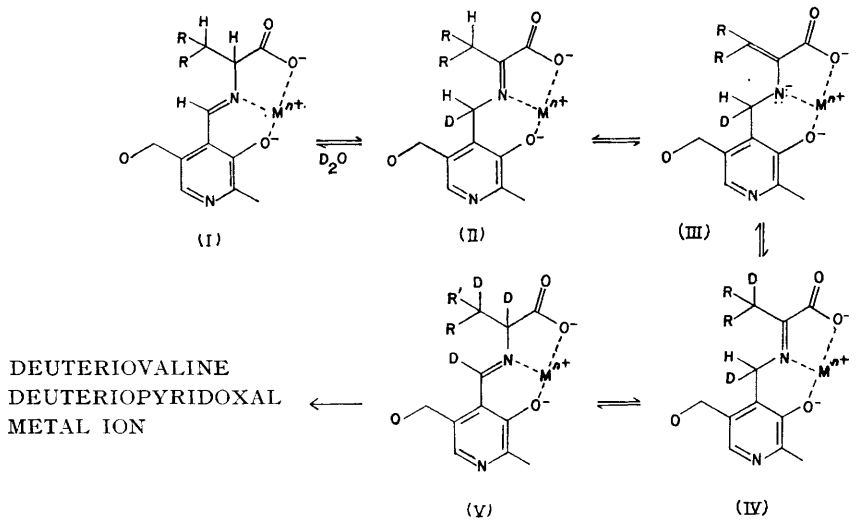
The following mechanism may now be proposed for β-proton exchange of α-amino-acids and α-oxo-acids. In this mechanism, the metal ion not only stabilizes the complex through chelation, but also acts as a positive centre to promote the electron shift in the ketimine chelate which results in dissociation of the β-proton.

A proton-catalysed reaction would also be expected to occur in a manner similar to the metal ion-catalysed scheme indicated here. Such a mechanism would account for the increase in the rate of β-exchange observed in the metal-free pyruvate–Schiffs base system. For one of the

† We have recently shown that the rate of enolization of a α-oxobutyrate is increased at least 100-fold by the addition of an equimolar amount of pyridoxamine and that this reaction necessarily goes through the Schiffs base and not through a free oxo-acid pathway.

steps (II) → (III) of this mechanism, the replacement of the metal ion by a proton would give a mechanism similar to that suggested by Bender for the amine-catalysed enolization of dihydroxyacetonephosphate.[6] Such a proton-catalysed reaction would have limited applicability, however, since the conditions favouring the protonation of the Schiffs base nitrogen will also result in the decomposition of the Schiffs base.

provides a general method for hydrogen exchange in a wide variety of amines and carbonyl compounds. This mechanism provides the first explanation of the observation by Junk and Svec[7] that deuterium was introduced into the β-position of leucine in the presence of pyridoxal and aluminium ion. Metal catalysis should be possible when a Schiffs base is formed between the amine and carbonyl functions. Metal catalysis would be



Mechanism of β- (and α-) deuteriation of α-amino-acids, α-oxo-acids, and pyridoxal pyridoxamine.

For Zn catalysis, $n = 2$; for Al catalysis, $n = 3$; for valine, R = Me_3; for alanine, R=H

These experiments indicate that the β-position of oxo-acids can be strongly activated in the presence of pyridoxamine either with or without the addition of metals. Since many vitamin B_6-catalysed reactions occur at the β-position, the imine–enamine tautomerism previously described should represent an important step for extending the catalytic effect of vitamin B_6 into the amino-acid or oxo-acid backbone.

The metal-catalysed mechanism now proposed expected to be particularly strong, as has been observed in our work, when either the carbonyl or amino-compound, or both, have an additional co-ordinating group to stabilize the intermediate chelate compound.

Further work is in progress to elucidate the characteristics of this reaction and its relevance to vitamin B_6 chemistry.

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