A Novel Threonine Aldolase Model

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Summary The Al^{III}-pyridoxal-catalysed threonine aldolase model reactions of threonine and of a new substrate, β-hydroxyvaline, are followed by quantitative n.m.r. measurements in D_2O solution; formation of acetone in the β-hydroxyvaline reaction system provides the first quantitative evidence for C–C bond fission of an α-amino-acid having an α-proton in the presence of pyridoxal and metal ions.

THE ^1H n.m.r. spectra of Al^{III} and Zn^{II} chelates of Schiff's bases formed by the reaction of pyridoxal with serine and threonine, as well as that of a synthetic amino-acid, β-hydroxyvaline, were investigated as models for the amino-acid aldolase reaction, and the ^1H n.m.r. resonances of the complexes have been assigned. The progress of the aldolase reaction was followed by quantitative measurement of the appropriate aldehyde or ketone resonances in D_2O. Figure 1 shows the methyl region of the n.m.r. spectra of a D_2O solution that is 0·10 M in pyridoxal, 0·10 M in threonine, and 0·05 M in aluminium(III). The disappearance of threonine and the appearance of acetaldehyde can be readily observed. It is difficult quantitatively to follow the formation of acetaldehyde since it undergoes aldol condensation under the conditions employed.

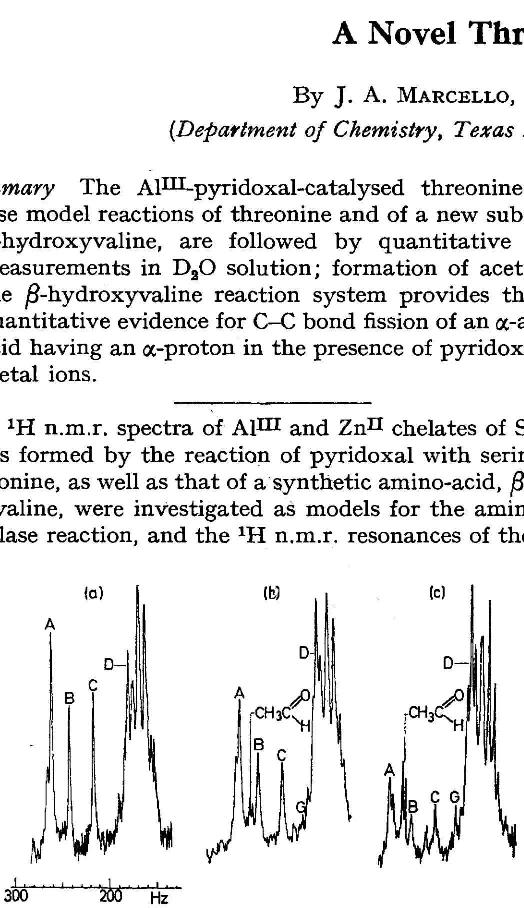


FIGURE 1. Changes in the methyl region of the 100 MHz n.m.r. spectra of a solution 0·10 M in DL-threonine, 0·10 M in pyridoxal, and, 0·050 M in Al^{III} at pD 9·30, showing the slow appearance of acetaldehyde and of bis(pyridoxylideneglycinato)aluminium(III) (G): a, initially; b, after 30 min; c, after 90 min; all at 30 ± 2 °C. Resonances are in Hz relative to hexamethyldisiloxane. A, B, C, D are the 2-methyl resonances of the pyridine ring, described previously.

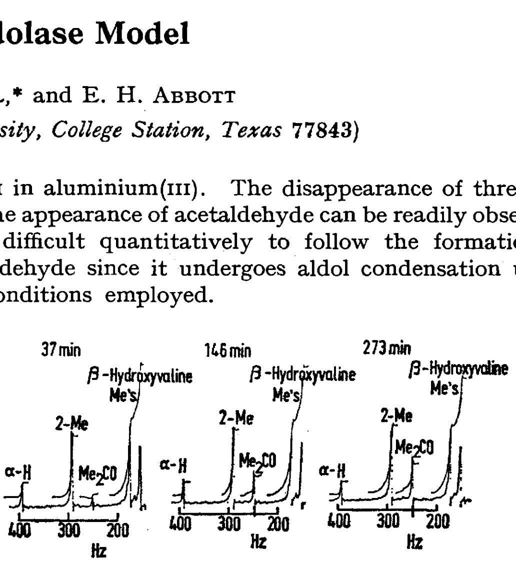


FIGURE 2. 100 MHz spectra of a D_2O solution 0·20 M in pyridoxal, 0·2 M in β-hydroxyvaline, and 0·10 M in Al^{III} at 30 ± 2 °C at pD 4·13. Resonances are in Hz relative to hexamethyldisiloxane.

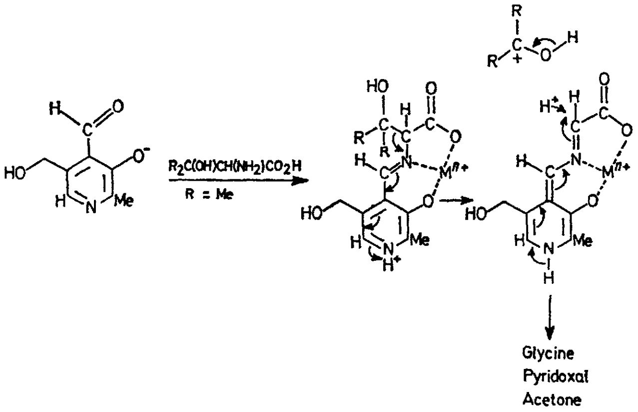
Because of the difficulties encountered in quantitatively following the appearance of the aldehyde, β-hydroxyvaline was used in place of threonine. The resultant products of this amino-acid in the model system are glycine and acetone, the latter being stable to aldol condensations.

The n.m.r. spectra from the reaction of 0·20 M pyridoxal, 0·20 M β-hydroxyvaline, and 0·10 M aluminium(III) (Figure 2) show clearly the formation of acetone and the disappearance of the β-hydroxyvaline. The effect of the pyridoxal is catalytic in that quantitative amounts of acetone were formed even though Schiff's base formation was less than 20% and the reaction was run at low pD.

Two mechanisms[1,2] have been suggested for these model systems. The Snell mechanism[1] (Scheme) includes direct cleavage of the αβ carbon–carbon bond in the Schiff's base chelate. Although prior model studies had revealed the formation of acetaldehyde,[3] a quantitative kinetic study in which reactants and products were fully accounted for had not been previously carried out. The observation[3] that α-methylserine reacts to give formaldehyde and α-alanine under the same conditions that serine and threonine form formaldehyde and acetaldehyde indicates that direct carbon–carbon fission occurs when there is no α-proton on the amino-acid unit. It does not prove, however, that the threonine model aldolase reaction does not take place, at least in part, through an α-deprotonated intermediate. Such an alternative or parallel route remains a strong possibility because of the occurrence of competing transaminations and racemizations in the serine and threonine Schiff's base system.

The reaction of the β-hydroxyvaline with the pyridoxal metal chelate gives clear evidence favouring direct carbon–carbon cleavage, since the intensity of the α-proton resonance (Figure 2) remained the same throughout the reaction, indicating that the α-proton had not dissociated during the reaction. If the α-proton had been dissociated, or had undergone exchange, [$^{2}H_{2}$]glycine would be expected to be formed, and the intensity of the α-proton n.m.r. peak would have decreased as the reaction progressed. The α-proton peak broadens slightly since, as the reaction proceeds, the observed peak is a combination of the α-proton resonances of both the deuterioglycine and the β-hydroxyvaline chelates.

The integrated sum of the methyl resonances of the amino-acid and of acetone remains constant throughout the reaction (Figure 2), indicating the absence of competing reactions such as transamination. We suggest that two factors contribute to the unique single, clean reaction in this pyridoxal-catalysed system:[1] the unusually low pH, which is considerably different from the optimium pH values of possible competing reactions,[3] and the presence of two methyl groups on the β-carbon atom of the amino-acid, which would be expected greatly to assist the rate-determining electron shift indicated in the Scheme.



Scheme

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