Metal Ion- and Pyridoxal-catalysed Transamination and Dephosphonylation of 2-Amino-3-phosphonopropionic Acid. A New Phosphonatase Model

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Summary Vitamin B_6 and metal ion-catalysed transamination of 2-amino-3-phosphonopropionic acid (APP) results in initial formation of the metal chelate of the pyridoxal–APP Schiff base, followed successively by

transamination to the ketimine chelate, dephosphonylation, reverse transamination, and finally hydrolysis to alanine and pyridoxal.

ALTHOUGH aminoalkylphosphonic acids are now recognized as important constituents of biological systems,[1] very little is known of their chemical reactions and biological functions. This paper reports the first metal ion- and pyridoxal-catalysed transamination and dephosphonylation of an aminophosphonic acid, 2-amino-3-phosphonopropionic acid (APP).

The electronic spectra of pyridoxal (PL) and aminomethylphosphonic acid (AMP) or 2-aminoethylphosphonic acid (2-AEP) in the absence and in the presence of Al^{III}, Cu^{II}, and Zn^{II} ions gave evidence[2] for initial Schiff base (aldimine) formation, and demonstrated the absence of transamination to the corresponding ketimine Schiff bases or to transamination products. These results showed that the equilibrium position of the isomerization aldimine $\rightleftharpoons$ ketimine lies far to the left, and the amount of ketimine formed is too small to be measured spectrally.

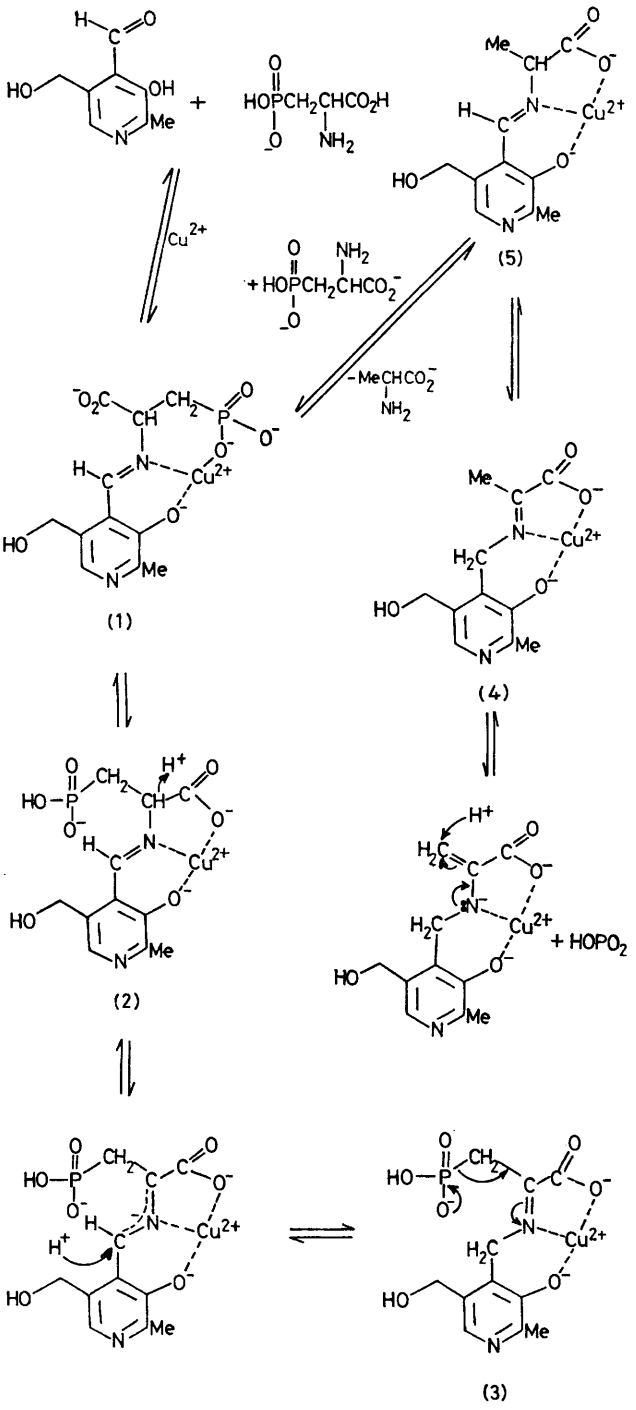
Solutions of APP containing catalytic (*ca.* 100 times lower concentration) amounts of Cu^{II} and PL at room temperature in the pH range 5—9 undergo aldimine chelate formation over a period of a few hours, as evidenced by the growth of an absorption band at 380 nm, and decrease in intensity of the free pyridoxal peak at 317 nm. Over 24 h the aldimine chelate band then decreases in intensity and the free pyridoxal peak shifts to that of pyridoxamine, λ_{max} 326 nm, providing clear evidence for transamination.

Analysis of the reaction system showed that while pyridoxal is lost, the total amount of pyridoxal and pyridoxamine remains constant. Continuous formation of inorganic phosphate indicates that the product of transamination undergoes subsequent dephosphonylation. Solutions containing APP alone, APP and PL without metal ion, and APP with metal ion, show no phosphate formation. Thus all three components are required for the dephosphonylation of APP. An important observation is that the amount of phosphate formed is significantly greater than the initial concentration of PL, which would be the limiting reactant for a stoicheiometric reaction. Formation of alanine as the final product was followed by paper chromatography (using ninhydrin), and confirmed with a Beckman amino-acid analyser.

A mechanism that accounts for the species formed in solutions containing PL, APP, and Cu^{II} ion is indicated in the Scheme. Transamination of (**2**) to (**3**) is followed by loss of the phosphonate group from the ketimine to form the pyruvate ketimine (**4**). Formation of alanine requires reverse transamination to the aldimine (**5**). While the C–P bond of aminophosphonic acids is normally very stable, in the ketimine with a β-phosphonate function as in (**3**) there is a metal ion-activated electronic pathway which, with negative charge on the (dissociated) phosphonate group, leads to C–P bond fission and release of metaphosphate.

Similar studies with Al^{III} and Zn^{II} in place of Cu^{II} showed the relative catalytic effects for these vitamin B_6-catalysed reactions to be $Al^{III} > Cu^{II} > Zn^{II}$.

In the case of APP no enzyme has been reported that catalyses a β-dephosphonylation reaction analogous to that illustrated in the Scheme. The logical nature of the reaction mechanism shown, and the analogy to β-decarboxylation mechanisms, suggests that enzymes that catalyse such β-dephosphonylation reactions probably do exist, and



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that transamination may be an essential step in the reaction pathway leading to C–P bond fission in biological systems.

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