Chemical Biological Methods for Understanding the Mechanism of Sugar-Processing Enzymes

Nature **1965**, *206*, 757; *Proc Natl Acad Sci USA* **1967**, *57*, 483; *Nature* **2001**, *412*, 835

Introduction & Hypothesis

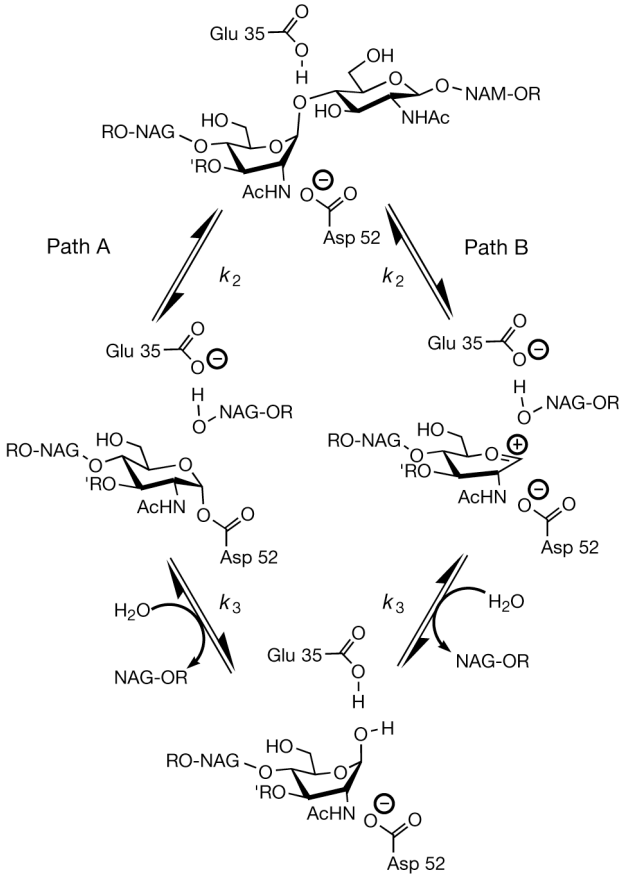
• Lysozyme HEWL is an enzyme found in large quantities in hen egg white. It degrades peptidoglycan in the cell wall of gram positive bacteria. The key residue it acts upon is an *N*-acetyl glucosaminide and so HEWL is a glycosidase (see **M3**)

• HEWL was the first protein for which a high resolution (2Å) x-ray structure was determined and in 1967 a mechanism was proposed by Phillips that invokes a glycosyl oxonium intermediate consistent with an S_N1 mechanism for the hydrolysis of an acetal (Path B).

• However such oxoniums are shortlived and so many have speculated as to whether such a true cationic intermediate could really be intercepted directly by water. It has been proposed that the cation is stabilized as an ion-pair with eg a Glu side chain.

• Other mechanisms (**M3**) can be proposed (eg Path A) that involve a glycosyl-enzyme intermediate.

• $k_H/k_D > 1$ which suggests that in the RDS/RLS a less crowded TS.



Withers and Davies used a mixture of intermediate trapping, mutant enzymes and crystallography to see if they could trap the intermediate and prove its structure.

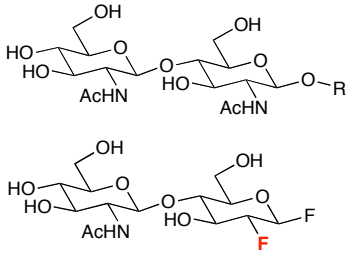
Method Development

• If Path A was correct then it should be possible to trap the intermediate by slowing down the 2nd step – the hydrolysis of the glycosyl ester by water. 2 approaches were tried:

i) using a mutant enzyme E35Q (but is similar in size of side chains) that lacks the general acid/base – without the GB then the rate of water attack (k_3 step) would be lowered

ii) using an altered sugar substrate that will create a less reactive glycosyl ester that will react more slowly with water in the k_3 step, even with the GB present.

• A 2-fluoro-2-deoxy derivative probe was designed. The electronegative F destabilizes the TS (which has significant developed positive charge) both to and (more importantly) from the glycosyl-enzyme intermediate. The anomeric F however compensates in the first step by providing a better leaving group than OR.



normal substrate type

probe designed to trap intermediate

Trapping of the Intermediate

• Mass spectrometry of the enzymes and probes and substrates was used to see if trapping could be observed.

• The use of both mutant and probe was needed to slow k_3 step enough to allow a high-enough steady state occupation for crystallography.

• The structure determined not only shows the intermediate but shows how formation of this resulting glycosyl enzyme intermediate requires dramatic conformational boat->chair movement from the Michaelis complex.