Modeling and Applications of Nitrile Hydratase

Rachel C. Linck Literature Seminar October 17, 2000

Nitrile hydratase (NHase) catalyzes the conversion of nitriles to the corresponding amides.[1] The enzyme contains either a non-heme low-spin Fe(III) or non-corrinoid low-spin Co(III) metal at its active site. The S_2N_3O ligand sphere (Figure 1) consists of three cysteines, two of which are post-translationally modified to sulfenic and sulfinic acids.[2] The nitrogen ligands are the peptide backbone amide nitrogens of a cysteine and a serine residue. The oxygen ligand is either a hydroxy group or a coordinated water molecule. The Fe(III)-containing enzyme has been characterized in its active[3] and inactive[4] forms by x-ray crystallograpy. NHase is regulated by NO, the first example of such a mechanism in an enzyme.[4] Although much is known about the structure of NHase, the mechanism by which it converts nitriles to amides is unclear.[3]

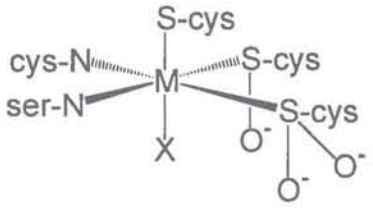

Figure 1

Synthetic models have been invoked in an attempt to determine the mechanism of nitrile hydrolysis by NHase. Kovacs has created several structural models of the enzyme.[5, 6, 7] These models exhibit spectroscopic signatures like the enzyme and limited reactivity; however, none of these models display reactivity with nitrile substrates.

Mascharak has successfully synthesized a series of structural models,[8, 9, 10] one of which also exhibits reactivity with nitriles (Figure 2).[11] The model, $[Co^{III}(PyPS)(OH)]^{2-}$, has *cis*-thiolate ligands, two carboxamido ligands, another nitrogen ligand, and a hydroxy group. The model completes 15 turnovers in 2 hours, and 18 turnovers in 4 hours at 50 °C with acetonitrile as the substrate.

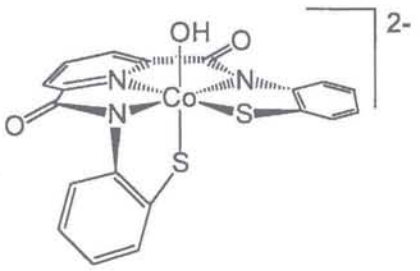

Figure 2

Acrylamide production occurs on an industrial scale of about 200,000 tons per annum.[12] One industrial process for acrylamide production involves the use of a Cu^{II} catalyst. This method is fraught with difficulties: high rate of acrylic acid formation, polymerization of acrylamide, and high reaction temperatures.[13] In 1981, the first bioreactor for the production of acrylamide using an Fe(III) strain of NHase as the catalyst was introduced. More than 30,000 tons per annum of acrylamide are currently prepared by this method, using a Co^{III} strain of NHase. This reaction allows milder conditions and fewer steps, thus reducing the cost of production.[13]

In addition, an immobilized enantioselective NHase preparation has been used in organic reactions to achieve selective stereochemistry. NHase ensures greater than 99% conversion to the preferred stereochemistry (Figure 3).[14, 15]

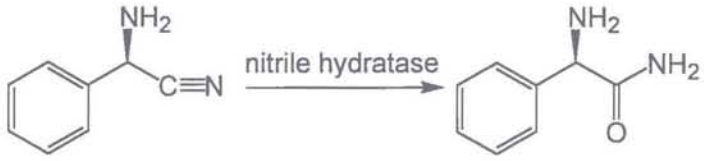

Figure 3

Future research in this field seeks to determine the mechanism of nitrile hydrolysis by NHase, to increase productivity in the industrial production of acrylamide, and to demonstrate the enzyme's utility in stereospecific synthetic chemistry.

References

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