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Research review paper

Nitrile hydratases (NHases): At the interface of academia and industry

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ABSTRACT

Nitrile hydratase (NHase, EC 4.2.1.84) is one of the key enzymes of nitrile metabolism in a large number of microbes that catalyses the hydration of nitriles to corresponding amides, and has been successfully adopted in chemical industry for production of acrylamide, nicotinamide and 5-cyanovaleramide. However, NHase is still under active consideration of enzymologists to expand its potential for synthesis of various amides. Most of the NHases have been reported for their limited substrates acceptability, low enantioselectivity and thermostability and therefore a considerable improvement is required for developing as robust biocatalyst for synthesis of a range of organic amides. Studies on biochemical properties, gene configuration, active-site chemical models and site-directed mutagenesis have given the insight into the structural and functional characteristics of NHase. Keeping in view, the present review critically describes the available information on natural sources (based on activity and phylogenetic analysis), biochemical properties, catalysis–structure relationship, molecular expression and potential applications of this enzyme.

Contents

1. Introduction

Nitriles are widely used starting materials and intermediates in organic synthesis. Hydration of nitriles to corresponding carboxamides is an important reaction in nature and organic synthesis. There are two approaches for hydration of nitriles i.e. chemical hydration (Jenes and Trogler, 1986) and enzymatic hydration by nitrile hydratase (NHase). This enzyme was first discovered from the bacterium *Arthrobacter* sp. J-1 and named as 'nitrile hydratase' with experimental data by Asano et al. at the Department of Agricultural

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Chemistry, University of Kyoto, Japan (Asano et al., 1980, 1982a,b). Later, *Arthrobacter* sp. J-1 was identified as *Rhodococcus rhodochrous* J1 (Kato et al., 2000). Asano et al.'s early work on the conversion of acrylonitrile to acrylamide with this enzyme indeed had high impact in academia and industry vis-a-vis it revolutionized the world enzyme engineering as new route involving a biocatalyst for the synthesis of acrylamide was reported. In due course of time enzymatic hydration of nitriles emerged as very efficient and preferred route for conversion of nitriles to corresponding amides over the chemical hydration of nitriles. A technical comparison of enzymatic and chemical hydration of nitriles is presented in Table 1. The discovery of NHase can be considered as boom in organic synthesis of amides since at the time of its discovery chemists were struggling for improving the existing chemical hydration reaction to achieve high yield of pure amides (Asano et al., 1980, 1982a,b). Before the discovery of NHase, the formation of acetamide was noticed in the reaction involving enzymatic hydrolysis of acetonitrile with whole cells of *Corynebacterium* (Fukuda et al., 1971) and *Nocardia rhodochrous* LL100-211 (Digeronimo and Antoine, 1976), however, the enzyme system responsible for this conversion of nitrile to amide was not known. The application of NHase in the synthesis of amides addressed most of the problems encountered in chemical hydration of nitriles (Table 1). Currently, the production of various amides (acrylamide, nicotinamide and 5-cynovaleramide, etc.) is being carried out at industrial scale using this enzyme and synthesis of a large number of other amides through this biocatalytic route is being extensible explored (Kobayashi et al., 1992; Thomas et al., 2002; Shaw et al., 2003; Hann et al., 1999; Wang, 2005).

The history of NHase since its discovery to date is the journey of this enzyme from academia to industry. In all these years ecological, microbiological, biochemical and molecular aspects of NHase have been studied in detail and from time to time available information have been reviewed. Most of the previous reviews on NHase invariably converse either the production of acrylamide, nicotinamide or its structure and gene expression of few cases (Yamada and Kobayashi, 1996; Kobayashi and Shimizu, 1998; Kobayashi and Shimizu 2000; Endo et al., 2001; Banerjee et al., 2002; Wang, 2005). However, there is no comprehensive review that critically scans information on NHases with a focus on its catalytic properties, molecular characteristics and mechanism of catalysis. This review summarises hitherto available information especially on NHase sources, catalytic features, molecular properties, mechanism of catalysis, heterologous/homologous expression and its potential biotechnological applications. It also discusses challenges and opportunities for future studies on NHases.

Table 1
A technical comparison of chemical and enzymatic hydration of nitrile to corresponding amides.

Parameter	Chemical hydration	Enzyme hydration
Catalyst	Acid or Base	Nitrile hydratase (NHase): A metallo-enzyme
Source of catalyst	Chemical synthesis	Active expression of NHase gene
Reaction	In highly acidic or basic aqueous media at 200–300 °C, under high pressure	In aqueous media (i.e. physiological buffer of pH neutral to slightly basic) at mild or low temperature (<30 °C)
Substrate selectivity	Broad-range without chemo-, stereo-, regio-selective	Narrow-range with chemo-, stereo-, and regio-selective
Recycling of catalyst	Not possible[a]	Possible by immobilization of biocatalyst
Extraction and purification of product	Organic-phase extraction and multi-step purification of product to remove the trace of sub products (organic acid) and by-products (carbon monoxide, HCN, etc.).	Organic-phase extraction, no requirement of extra-purification due to complete conversion of substrate and no by-product formation
Yield	Low	High

[a] Sometimes possible in heterogeneous transition metal catalyst.

2. NHase in nitrile-metabolising pathway

Biotransformation of nitriles follows two routes to produce organic acids (Asano et al., 1986). In first route, nitriles undergo a direct hydrolysis to form the corresponding carboxylic acids and ammonia by the action of nitrilase (EC 3.5.5.1) (Thimann and Mahadevan, 1964a,b; Harper, 1977). In the second route nitriles are first hydrated into corresponding amides by NHase (Asano et al., 1980) and subsequently amides are hydrolyzed to carboxylic acids and ammonia by action of amidase (EC 3.5.1.4). These three enzymes (nitrilase, NHase and amidase) are the key catalysts in the nitrile metabolism as shown in Fig. 1.

Usually, the microbes contain either a nitrilase (Robinson and Hook, 1964; Harper, 1977; Harper, 1985; Kobayashi et al., 1990; Nagasawa et al., 1990; Layh et al., 1998; Prasad et al., 2007a) or NHase-amidase system (Martinkova and Kren, 2002; Prasad et al., 2005) for the hydrolysis of nitriles. However, some microorganisms such as *R. rhodochrous* J1 (Bandyopadhyay et al., 1986; Mathew et al., 1988; Mauger et al., 1988), *R. rhodochrous* LL 100-21 (Linton and Knowles, 1986), *R. rhodochrous* PA-34 (Bhalla et al., 1992, 1995; Prasad et al., 2009) and *N. globerula* NHB-2 (Bhalla and Kumar, 2005) contain both nitrilase and NHase-amidase systems. These three enzymes of nitrile hydrolysis can be induced selectively by substitution of one of the nitrile or amide (i.e. its substrate or product or their analogue) in enzyme expression media. Based on some simple experiments, it can be easily worked out whether the given nitrile metabolising organism has one of or both the nitrile hydrolysing route as mentioned above (Mathew et al., 1988; Nagasawa et al., 1991a; Wieser et al., 1998; Bhalla and Kumar, 2005). Prasad et al. (2007a) expended this study for systematic characterisation of nitrile-hydrolysing enzyme system in *Rhodococcus* sp. NDB1165. The metabolism of nitriles through route first or second is determined by using nitriles/amides as substrate and analysis of the subsequent products. The major product in the first route of nitrile metabolism involving nitrilase is invariably the corresponding carboxylic acid of the nitrile substrate, however, some nitrilases have also been reported to produce amide from the nitrile (Fernandes et al., 2006; Martinkova and Kren, 2010), but certainly the nitrilases do not catalyse the conversion of amides to acids. Further, the expected enzyme system can be confirmed by purification of enzyme/s and identification of corresponding gene/s by southern hybridization and polymerase chain reaction (Asano et al., 1982a,b; Nagasawa et al., 1991b; Wieser et al., 1998; Ikehata et al., 1989; Nishiyama et al., 1991; Kobayashi et al., 1991; Petrillo et al., 2005; Precious et al., 2001).

3. Natural sources and phylogenetic analysis of NHase

A large number of microbes belonging to various species of following diverse genera of Proteobacteria such as *Acidovorax* (Gavagan et al., 1999), *Agrobacterium* (O'Grady and Pembroke, 1994; Vosahlova et al., 1997; Wieser et al., 1997; Bauer et al., 1994; Kobayashi et al., 1996), *Bacillus* (Kim and Oriel, 2000; Pereira et al., 1998; Cramp and Cowan, 1999; Takashima et al., 2000), *Bradyrhizobium* (Vega-Hernández et al., 2002), *Burkholderia* (Shoemaker et al., 2001), *Comanonas* (Stevens et al., 2003; Petrillo et al., 2005), *Klebsiella* (Nawaz et al., 1991), *Mezorhizobium* (Feng et al., 2008), *Moraxella* (Anton et al., 1997), *Pantoea* (Hensel et al., 2002), *Pseudomonas* (Yanase et al., 1985; Masutomo et al., 1995; Nitto Chem, 1992; Yamada et al., 1980; Yamada 1987; Anton et al., 1997; Wu et al., 1997; Di-Cosimo et al., 1998; Zhao et al., 1995; Fallon et al., 1997), *Rhizobium* (Kobayashi et al., 1996), *Rhodopseudomonas* (Black et al., 2010), *Serratia* (Anton et al., 1997; Jin et al., 2010); Actinobacteria such as