Biocatalytic Hydrolysis of Nitriles

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Abstract—Two pathways of enzymatic hydrolysis of nitriles to carboxylic acids are known today. Under the action of nitrilases, nitriles turn into carboxylic acids in a single step via the addition of two water molecules. Under the action of nitrile hydratases, nitriles turn into amides, which are then hydrolyzed by amidase to carboxylic acids. This review deals with the structure, substrate specificity, mechanisms of action, and industrial potential of these three enzymes. Examples of successful use of the nitrile-hydrolyzing enzymes in the large-scale manufacture of acrylamide and nicotinamide in Russia and abroad and in the industrial synthesis of α-hydroxy acids (glycolic and R-mandelic acids) are presented. The stereoselectivity and regioselectivity of the enzymes make them useful in the synthesis of chiral synthons for the production of important pharmaceuticals (statins, antimitotic agents, and enzyme inhibitors).

Keywords: enzymatic hydrolysis of nitriles, nitrile hydratases, amidases, nitrilases, use in organic synthesis.

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1. INTRODUCTION

Nitriles, or organocyanides (R–CN), are widely spread in nature. They are synthesized by plants and serve as precursors of hormones (phenylacetonitrile), storage compounds (cyanoglycosides and cyanolipids), etc [1].

Synthetic nitriles are widely employed in organic synthesis as precursors of various amides and acids. The conventional chemical hydrolysis of nitriles requires severe reaction conditions (acidic or alkaline pH and temperature above 100°C) and is accompanied by the formation of undesired by-products and large amounts of waste. An alternative to this process is biocatalytic nitrile hydrolysis, which takes place under...
mild conditions (neutral pH and room temperature), selectively involves only nitrile groups, and is in some cases stereoselective and/or regioselective, which is particularly significant in the synthesis of biologically active compounds.

Two natural pathways are presently known for nitrile hydrolysis to carboxylic acids. The first pathway includes the successive action of two enzymes, namely, nitrile hydratase (EC 4.2.1.84) and amidase (EC 3.5.1.4). Nitrile hydratase adds one water molecule to the nitrile, converting it into an amide, and amidase hydrolyzes the amide to an acid (Scheme 1). The second pathway is the single-step conversion of the nitrile into an organic acid via the addition of two water molecules catalyzed by nitrilase (EC 3.5.5.1), as is shown in Scheme 1. All of these enzymes—nitrile hydratases, nitrilases, and amidases—have recently attracted considerable attention from researchers and synthetic chemists as biocatalysts for organic syntheses. Over ten catalytic processes involving nitrile-hydrolyzing enzymes have already been commercialized to date.

The purpose of this review is to consider the current data on the mechanism of action of nitrile metabolism enzymes, their properties, and their use in the chemical and pharmaceutical industries.

2. ENZYMES CATALYZING NITRILE CONVERSION

2.1. Nitrile Hydratases

Nitrile hydratase was discovered for the first time in cells of *Rhodococcus rhodochrous* J1 bacteria (formerly called *Arthrobacter* sp. J1) in 1980 [2]. Although dozens of enzymes from various bacteria have been studied to date, *Rhodococcus* and other Actinomycetales are still the most widespread sources of new nitrile hydratases [3]. On the whole, the main reservoir of nitrile hydratases are prokaryotes, primarily bacteria. A computer-aided search for new nitrile hydratases in the genome sequences represented in public databases permitted to identify this enzyme for the first time in a eukaryotic organism, specifically, *Monosiga brevicollis* (UniRef database, UniProt identifier A9V2C1). However, analysis of the gene sequence suggests that there could be a horizontal transfer of nitrile hydratase genes from proteobacteria [4].

All hitherto studied nitrile hydratases have a common structure. They are heterodimeric proteins consisting of α and β subunits with a molar mass of 22 and 28 kDa, respectively. With increasing concentration, the αβ dimers often form tetramers and higher oligomers. Nitrile hydratases are metalloenzymes containing nonheme iron (Fe$^{3+}$) or cobalt (Co$^{3+}$) in their active sites. Accordingly, the nitrile hydratases are divided into two groups: iron-containing (Fe-type) and cobalt-containing (Co-type) enzymes. The nitrile hydratase types differ in substrate specificity and activity, although they are highly homologous in their amino acid sequences, particularly in the regions belonging to the active site. There are several enzymes containing other metals (Zn or Cu); however, there is no evidence that these metals are involved in catalysis [5, 6].

Fe-type nitrile hydratases have an interesting specific feature. The enzymes synthesized in bacteria grown in the dark are inactive and can be activated by visible light [7–9]. It was demonstrated by optical spectroscopy, chromatography, and mass spectrometry that nitrile hydratases in the inactive state contain a nitrogen monoxide molecule bound to the iron atom [10]. When exposed to visible light, nitrogen monoxide leaves the active site, and this is accompanied by local conformational changes and by the activation of the enzyme [11]. This is the earliest known case of regulation of a bacterial enzyme by nitrogen mon-