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Review

Selection and screening for enzymes of nitrile metabolism

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Abstract

This work critically reviews the assays of nitrile-converting and nitrile-forming enzymes (nitrilases, nitrile hydratases, amidases, aldoxime dehydratases). Most of the strains producing such enzymes were obtained by selection on media with nitriles, amides or aldoximes as nitrogen sources. Activity and enantioselectivity of the enzymes was usually assayed by time-consuming chromatographic analysis of substrates and the corresponding reaction products. Attempts at introducing faster assays resulted in several spectrophotometric methods for reaction product (ammonia, hydroxamate, methacrylamide, benzamide, etc.) determination. Recently, new methods for colorimetric and fluorimetric determination of ammonia have been developed, which appear promising for high-throughput assays. Alternatively, methods consisting in determination of NADH consumed in a coupled amination reaction or pH-responsive methods are promising for this purpose. All the above selection and screening methods establish fundamental conditions for the design of hierarchical screening projects. However, the potential of these principles, in particular spectrophotometric and fluorimetric methods, will be probably further exploited and adapted to multiwell plate and robotic systems.

Keywords: Nitrilase; Nitrile hydratase; Amidase; Aldoxime dehydratase; Selection; Screening

Contents

1. Introduction

The metabolism of compounds containing CN-group involves a broad spectrum of reactions, including enzymatic hydrolysis (see below), oxygenase/oxynitrilase-catalyzed pathways (Jallageas et al., 1980) and cyanide reduction by

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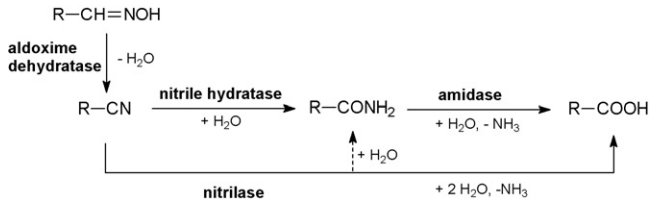

Fig. 1. Nitrile biotransformations catalyzed by enzymes reviewed in this work.

nitrogenase (EC 1.18.6.1) (Liu et al., 1997). The present review of screening and selection methods is primarily focused on nitrile hydrolysis, which proceeds by two distinct mechanisms, either a nitrile hydratase (EC 4.2.1.84)–amidase (EC 3.5.1.4) sequential reaction or a single-step process catalyzed by nitrilase (EC 3.5.5.1). The assays of aldoxime dehydratases (EC 4.99.1.5), which synthesize nitriles from aldoximes, are also discussed. Together with oxynitrilases (EC 4.1.2.10) (for instance, Johnson et al., 2000), all the enzymes involved in cascade conversions of aldoximes to carboxylic acids (see Fig. 1) gain growing interest as useful alternatives of conventional catalysts.

Moreover, their chemo-, stereo- and regioselectivity open a straightforward way to a broad variety of amides, carboxylic acids, amines and other intermediates of fine and pharmaceutical chemicals (for review see Sugai et al., 1997; Martínková and Křen, 2002). The past two decades have witnessed an intensive research into this field of biocatalysis, as illustrated by the growing number of articles dealing with enzymatic nitrile hydrolysis (see Fig. 2). The patent literature exhibits the same trend (see Sugai et al., 1997; Martínková and Křen, 2002), patents covering not only process design, but also enzyme improvement. The search for new enzymes with improved biocatalytic properties posed growing demands on the development of appropriate methods.

In general, assaying a large number of enzyme sources can be based on either screening or selection (Demirjan et al., 2000). Screening methods are largely readily available and quantitative, but every single colony must be analyzed for enzyme activity. Selection methods are based on using a selective medium, which allows only the colonies with the desired enzyme to grow. Therefore, they are more difficult to develop and provide only qualitative results, but have a very high throughput.

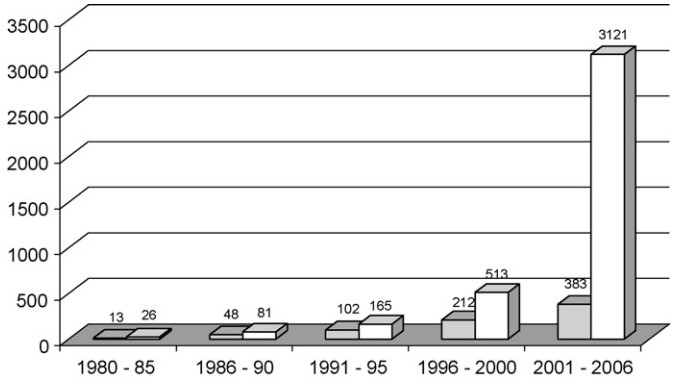

Fig. 2. Number of publications on enzymatic nitrile hydrolysis (according to www.scopus.com; nitrile hydratase or nitrilase or aldoxime dehydratase): grey, articles; white, patents.

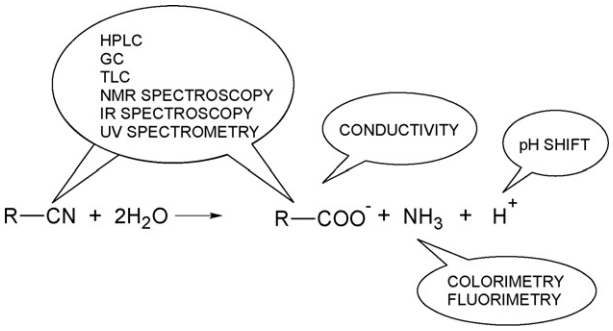

Fig. 3. Determination of products formed in a nitrilase-catalyzed reaction.

Selection has been routinely used to obtain nitrile- or amide-utilizing microorganisms (see Section 2). A broad spectrum of methods is applicable to assays of their enzymatic activities, but development of the corresponding high-throughput screens is a complex task. Different reaction products of a nitrilase-catalyzed reaction and possible methods of their quantitation are illustrated in Fig. 3.

Direct measurement of nitriles, amides and acids is not generally possible but methods based on UV spectrometry and NMR or IR spectroscopy have been applied to some specific cases (see Section 3.1.1.1). Therefore, reaction products have been most often separated by chromatographic methods prior to spectrophotometric quantitation (organic compounds) or determined in coupled reactions (ammonia). The frequency of use of these and other methods in a set of 111 articles is shown in Fig. 4.

Liquid or gas chromatography is useful for accurate analysis of the reaction products and especially for enantioselectivity assays, but unsuitable for high-throughput screens. Colorimetric or fluorimetric determination of ammonia as a common product of nitrilases and amidases forms a promising fundament for development of faster screening methods (see Section 3.2.2.1). However, these methods are not utilizable for nitrile hydratases and aldoxime dehydratases, unless another enzyme (amidase or nitrilase) able to release ammonia from the product (amide, nitrile) is added to the reaction mixture. Furthermore, numerous reactions catalyzed by crude enzymes can interfere with ammonia formation. Another significant drawback of these methods is their unsuitability for continuous assays. Therefore, *in situ*

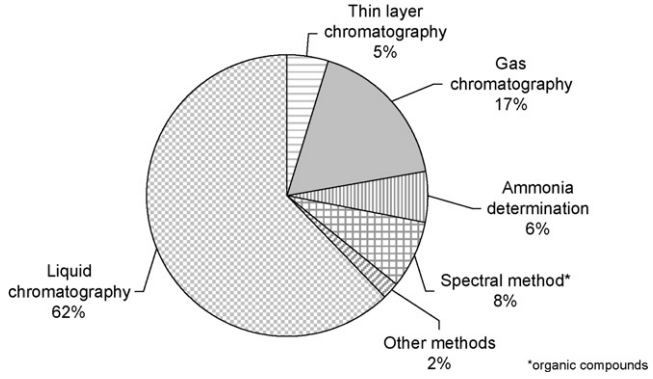

Fig. 4. Type of nitrilase and nitrile hydratase assays used in 111 articles published between January 2000 and February 2007 (according to www.scopus.com).