Identification and analysis of a hydrazide-degrading enzyme from mycobacteria

Ken-Ichi OINUMA

Department of Bacteriology, Osaka City University Graduate School of Medicine

Research aims

Hydrazine and its derivatives constitute an important group of compounds with tremendous industrial value, being used as dyes, adhesives, pharmaceuticals, herbicides, or precursors of these products. Although these compounds are known to exist in nature as secondary metabolites of microorganisms, their biosynthetic and degradation pathways are largely unknown. Acylated derivatives of hydrazine are called hydrazides. Several reports have described hydrazide-degrading enzymes. For example, Toida reported in the 1960s that *Mycobacterium avium*, a non-tubercular mycobacterium, produces an enzyme named hydrazidase that hydrolyzes the anti-tubercular agent isoniazid (INH)[1] (Fig. 1). Another report from the 1970s describes INH-hydrolyzing activity in *Mycobacterium smegmatis*[2]. These enzymes are important research subjects not only because of their possible involvement in INH resistance in mycobacteria but also because of their industrial value as biocatalysts. In the last several decades, however, no progress has been made in these studies, and the identity of these enzymes remains to be elucidated. In this study, we aimed to isolate and identify a hydrazidase from a mycobacterium and reveal its characteristics and distribution in nature.

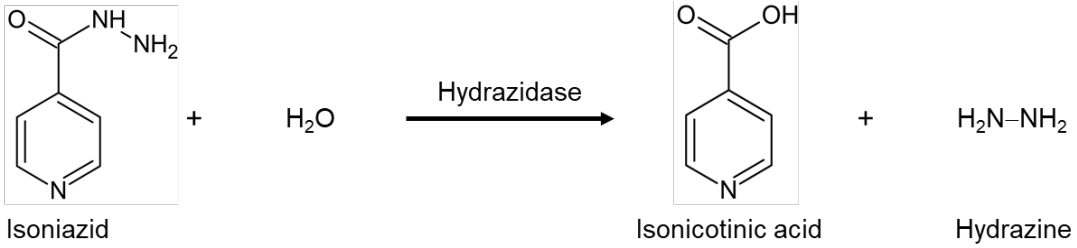

Fig. 1. Isoniazid-hydrolyzing reaction catalyzed by hydrazidase.

Methods

M. smegmatis MC^{2}155, a frequently used model strain for studying mycobacteria, was chosen as the subject for our study. An *M. avium* clinical isolate, obtained from the Osaka City University Hospital, and *Mycobacterium bovis* BCG Pasteur were used to investigate the distribution of INH-hydrolyzing activity among mycobacteria. These bacteria were grown using Middlebrook 7H9 or M9-based liquid media at 37 °C with shaking.

INH-hydrolyzing activity was measured by monitoring formation of isonicotinic acid using an ultra performance liquid chromatography system equipped with an ACQUITY UPLC BEH Amide column (2.1 × 100 mm) (Waters). Columns manufactured by GE Healthcare were used for protein purification. Identification of the purified enzyme was performed by peptide mass fingerprinting based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis following trypsin digestion.

Results

We first performed an enzyme assay using 7H9-grown *M. smegmatis* MC^2155 cells, which demonstrated the INH-hydrolyzing activity of this strain. We then tested the dependence of this enzyme activity on the composition of the growth media. Although the activity was low in 7H9-grown cells, it increased dramatically after incubating cells for 8 hours in an INH-containing synthetic medium (Fig. 2). Addition of glycerol and $MgSO_4$ to the synthetic media resulted in higher activity. We tried to purify the enzyme from cells grown under optimal INH-producing conditions. Using a combination of ammonium sulfate precipitation and several different strategies of column chromatography, we succeeded in purifying the enzyme to homogeneity (Fig. 3). Peptide mass fingerprinting analysis of the purified material revealed that the enzyme was an amidase named PzaA that has been known to hydrolyze pyrazinamide and nicotinamide [3]. These results demonstrate, for the first time, that PzaA is involved in INH degradation by *M. smegmatis*.

To investigate the distribution of hydrazidase among other mycobacteria, we measured the INH-hydrolyzing activity of an *M. avium* clinical isolate and *Mycobacterium bovis* BCG Pasteur. Both strains showed similar levels of activity as *M. smegmatis* MC^2155. We then tried to identify the enzyme using a BLAST search with the PzaA amino acid sequence as query. Although several amidase-like gene products were found, with sequence identities as high as 30-35% with PzaA, we could not conclusively identify the INH-hydrolyzing enzymes due to lack of strong candidates with prominently high homology.

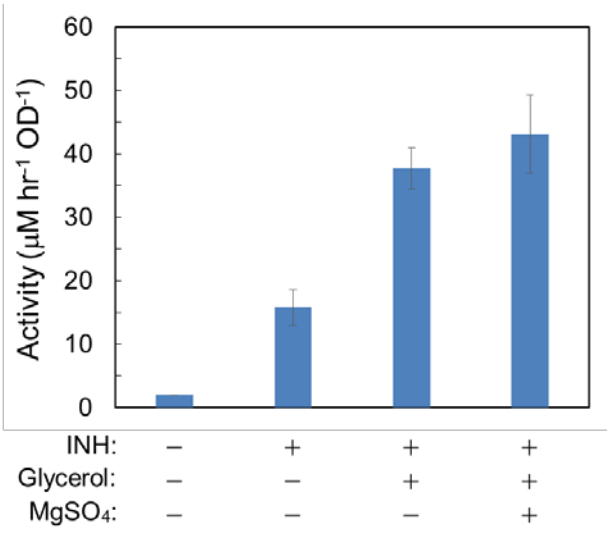

Fig. 2. Effect of synthetic media additives on the INH-hydrolyzing activity of *M. smegmatis*. *M. smegmatis* MC^2155 cells grown in 7H9 were transferred to synthetic media containing the indicated components and incubated for 8 hours. The activities of resting cells were normalized by optical density. Bars indicate standard deviations (n = 3).

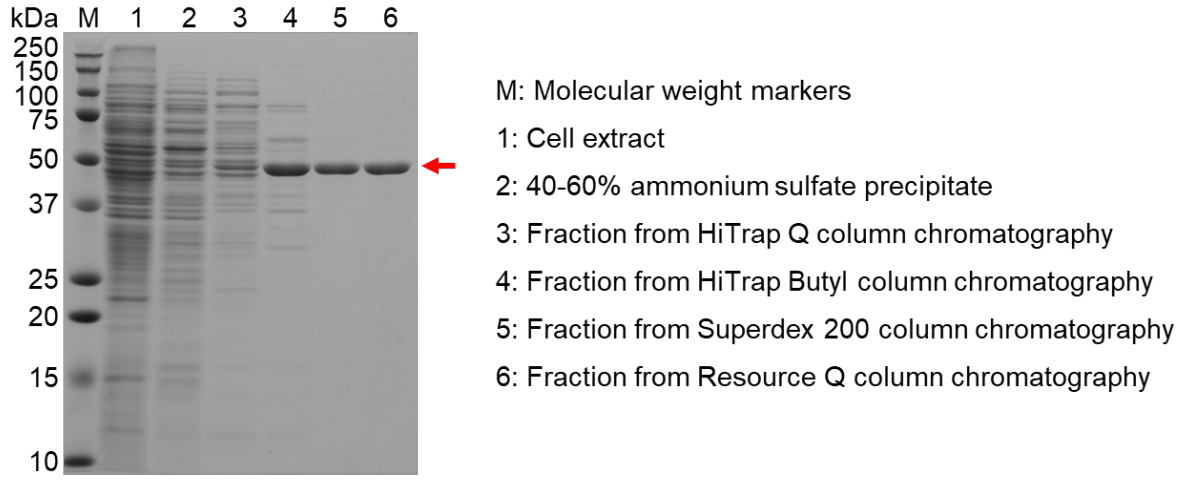

Fig. 3. Purification of the INH-hydrolyzing enzyme from *M. smegmatis* MC^2155. An image of a CBB-stained SDS-PAGE gel is shown. The arrow indicates the purified enzyme.

Conclusion

The INH-hydrolyzing enzyme of *M. smegmatis* has been identified as PzaA, revealing a previously unknown secondary function of this amidase as a hydrazidase. This is the first successful identification of a mycobacterial hydrazidase. Although we assume that enzymes similar to PzaA underlie the hydrazidase activities seen in other mycobacterial species, we could not identify them by homology searches of available genome sequences. Aiming at medical and zymological applications, we plan to continue this research, by including characterization of PzaA and identification of INH-hydrolyzing enzymes from other mycobacteria.

References

1) Toida, I. (1962) Isoniazid-hydrolyzing enzyme of mycobacteria. *Am. Rev. Respir. Dis.* **85:** 720-726.
2) Fishbain, D., Ling, G., and Kushner, D. J. (1972) Isoniazid metabolism and binding by sensitive and resistant strains of *Mycobacterium smegmatis*. *Can. J. Microbiol.* **18**: 783-792.
3) Boshoff, H. I. M. and Mizrahi, V. (1998) Purification, gene cloning, targeted knockout, overexpression, and biochemical characterization of the major pyrazinamidase from *Mycobacterium smegmatis*. *J. Bacteriol.* **180:** 5809-5814.