Biochemical and Biophysical Research Communications xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Improvement of stability of nitrile hydratase via protein fragment swapping

Youtian Cui [a,1], Wenjing Cui [a,1], Zhongmei Liu [a], Li Zhou [a], Michihiko Kobayashi [b,*], Zhemin Zhou [a,*]

[a] Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, People's Republic of China
[b] Institute of Applied Biochemistry and the Graduate School of Life and Environmental Sciences, The University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan

ARTICLE INFO

Article history:
Received 15 May 2014
Available online xxxx

Keywords:
Nitrile hydratase
Fragment swapping
Enzyme stability
Molecular dynamics
Homologous recombination

ABSTRACT

Nitrile hydratase (NHase), which catalyzes the hydration of nitriles to amides, is the key enzyme for the production of amides in industries. However, the poor stability of this enzyme under the reaction conditions is a drawback of its industrial application. In this study, we aimed to improve the stability of NHase (*Pp*NHase) from *Pseudomonas putida* NRRL-18668 using a homologous protein fragment swapping strategy. One thermophilic NHase fragment from *Comamonas testosteroni* 5-MGAM-4D and two fragments from *Pseudonocardia thermophila* JCM3095 were selected to swap the corresponding fragments of *Pp*NHase. Seven chimeric NHases were designed using STAR (site targeted amino recombination) software and molecular dynamics to determine the crossover sites for fragment recombination. All constructed chimeric NHases showed 1.4- to 3.5-fold enhancement in thermostability and six of them become more tolerant to high-concentration product. Notably, one of these NHases, 3AB, exhibited a 1.4 ± 0.05-fold increase in activity compared to the wild-type *Pp*NHase. Circular dichroism spectrum analysis and homology modeling revealed that the 3AB slightly differed in secondary structure from wild-type *Pp*NHase. The 3AB constructed in this study is useful for further industrial application, and the method for designing the chimeric protein using homologous protein fragment swapping without a decrease in activity may be a strategy to improve the stability of other enzymes.

1. Introduction

Hydration of nitriles to their corresponding amides is an important reaction that not only occurs in nature but also in organic synthesis. There are two approaches for the hydration of nitriles: enzymatic hydration and chemical hydration by a catalyst, such as copper [1]. Nitrile hydratase (NHase, EC 4.2.1.84), which catalyzes the hydration of nitriles to corresponding amides is one of the key enzymes of nitrile metabolism in a large number of microorganisms [1,2]. The enzyme, which consists of α- and β-subunits, contains either non-heme iron [3] or non-corrinoid cobalt in the active site [4]. NHase has also been successfully adopted in the chemical industry for the production of acrylamide, nicotinamide and 5-cyanovaleramide [1].

One of the drawbacks in the application of NHase is their poor thermostability and stability under high-concentration product. In the bioconversion process, the additional refrigeration to maintain the activity adds the costs of energy and the high accumulation of the product makes the enzyme inactive irreversibly [5]. Although an improvement in the stability of NHase is needed for its industrial application, successful cases of protein engineering of NHase are rarely reported until recently [6].

Many protein engineering strategies have been developed to improve the stability of enzymes and some certain enzymes have been redesigned and extended to the large-scale production [7]. Among these strategies, engineering using homologous recombination could harness structural and evolutionary information to design highly mutated, yet still natively folded, chimeric proteins and protein libraries [8]. Many proteins have been successfully modified using random or rational recombination methods [9,10]. For the recombinant method, it is crucial to select the corresponding recombination fragments and optimal recombination sites. In contrast to random recombination, site-directed recombination via the identification of specific sites in parental sequences in which their parts can be interchanged, enhances the successful rates of functional protein generation [11]. Several tools are available to identify optimal recombination sites [11,12]. Using these tools and swapping protein segments, such as domains, fragments

* Corresponding authors. Fax: +81 29 853 4605 (Institute) (M. Kobayashi). Fax: +86 510 85197551 (Z. Zhou).
E-mail addresses: kobay@agbi.tsukuba.ac.jp (M. Kobayashi), zhmzhou@jiangnan.edu.cn (Z. Zhou).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.05.127
0006-291X/

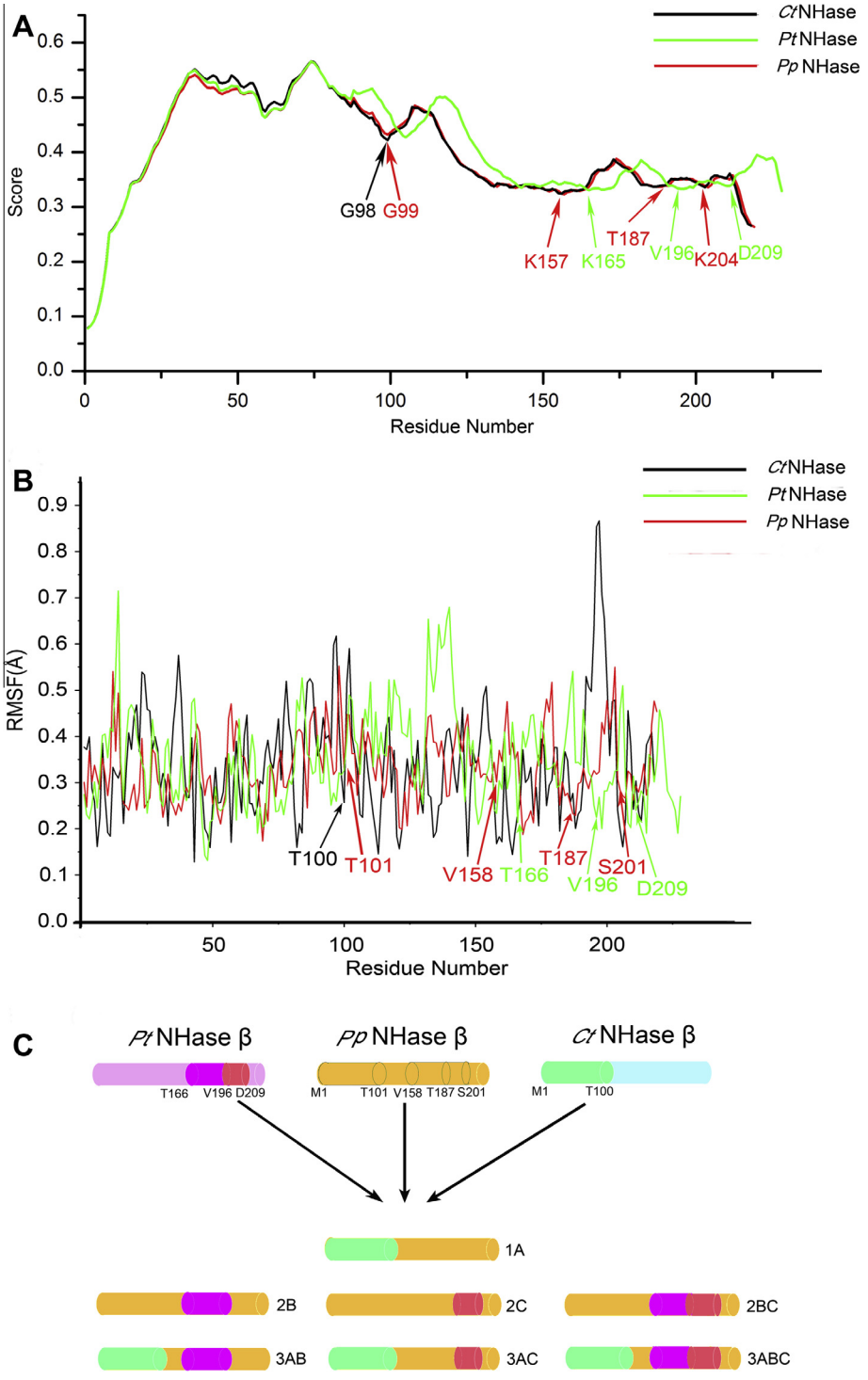

Fig. 1. STAR score profile, RMSF values for *Pp*NHase, *Ct*NHase and *Pt*NHase and schematic of chimeric NHases construction. (A) STAR score curves for NHases. The troughs with lower scores indicate the most potential recombination crossover sites without disturbing the structural stability. (B) RMSF obtained from 5-ns trajectories of *Pp*NHase, *Ct*NHase and *Pt*NHase. (C) Structural organization of chimeric NHases. The numbers indicate the parent NHases from which the fragments were selected. (1) *Ct*NHase; (2) *Pt*NHase; (3) both *Ct*NHase and *Pt*NHase. Wild-type *Pp*NHase is colored in orange. The fragments colored in red are from *Pt*NHase, and the one colored in green is from *Ct*NHase. Detailed organization of chimeric NHases are shown in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and termini, all of which stem from proteins with better qualities, could be utilized to relieve or eliminate the drawbacks of wild-type proteins.

In this study, NHase (*Pp*NHase) from *Pseudomonas putida* NRRL-18668 was modified to improve thermostability using homologous recombination. One thermophilic fragment from *Comamonas testosteroni* 5-MGAM-4D NHase (*Ct*NHase) and two fragments from *Pseudonocardia thermophila* JCM3095 NHase (*Pt*NHase) were selected to swap the corresponding fragments of *Pp*NHase, resulting in seven chimeric NHases. Although *Pp*NHase possess relatively poor stability *Ct*NHase and *Pt*NHase, it is notably that this enzyme has the higher activity, broad substrate specificity, demonstrated stereoselective, and regioselective capabilities [13,14]. The seven chimeric NHases exhibited higher thermostability than that of

Please cite this article in press as: Y. Cui et al., Improvement of stability of nitrile hydratase via protein fragment swapping, Biochem. Biophys. Res. Commun. (2014), http://dx.doi.org/10.1016/j.bbrc.2014.05.127