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ORIGINAL ARTICLE

# Highly Stable Mutant Bacterial Formate Dehydrogenase with Improved Catalytic Properties

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**Abstract**—$NAD^+$-dependent formate dehydrogenase (FDH, EC 1.2.1.2) from methylotrophic bacterium *Pseudomonas* sp.101 (PseFDH) has one of the highest thermal stability among all known enzymes of this group. The introduction of a number of amino acid substitutions into PseFDH made it possible to obtain a multipoint mutant PseFDH SM4S enzyme with even higher temperature and chemical stability. Previously, we showed that the introduction of additional single point replacements S131A, or S160A, or E170D into PseFDH SM4S led to further stabilization of the enzyme. In this work, based on the PseFDH SM4S S131A mutant, new mutant FDHs obtained, in which, compared to PseFDH SM4S, we added double S131A/E170D (M2), triple S131A/S160A/E170D (M3) and quadruple S131A/S160A/E170D/S145A (PseFDH SM4A M3) amino acids replacements. The new PseFDH mutants were overexpressed in *E. coli* cells, purified and characterized. The S131A/E170D and S131A/S160A/E170D changes provided further improving thermal stability. The introduction of the S145A substitution into PseFDH SM4A M3 leads to a significant decrease in $K_M^{NAD+}$ and $K_M^{HCOO-}$ while maintaining the catalytic constant at the same level. This mutant form can be successfully used in NADH regeneration systems, as well as for the detection of $NAD^+$ and formate in biological systems.

**Keywords:** formate dehydrogenase, *Pseudomonas* sp.101, catalytic properties, thermal stability, site-directed mutagenesis

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## INTRODUCTION

$NAD^+$-dependent formate dehydrogenase (FDH, EC 1.2.1.2) from the methylotrophic bacteria *Pseudomonas* sp. 101 (PseFDH) has been actively studied since the early 1970s. In 1977, this enzyme was obtained in purified form and its main properties were studied [1]. PseFDH is the first bacterial formate dehydrogenase whose gene was cloned and successfully expressed in *E.coli* cells [2, 3]. Although a large number of new FDHs from various sources have been described over the past 50+ years, PseFDH still remains the enzyme with the highest thermostability among the enzymes of this group [4]. In addition, like most bacterial formate dehydrogenases, PseFDH has a higher catalytic constant compared to eukaryotic FDHs [5, 6]. A number of crystal structures have been obtained for PseFDH both in free form (2NAC, 2GO1) and in complex with $NAD^+$ and formate (2NAD, 2GUG).

In our laboratory systematic studies on the structure-function relationship as well as work to improve the properties by rational design have been carried out for PseFDH and formate dehydrogenases from other sources [5–7]. Experiments have been performed to identify key amino acid residues for catalysis and substrate channel structure [8–10]. Amino acid substitutions (single and multipoint) were made, resulting in improved chemical [11–13] and temperature stability of PseFDH [14–16]. PseFDH is also the first formate dehydrogenase whose coenzyme specificity was changed from $NAD^+$ to $NADP^+$ [17, 18]. Mutant forms with an altered isoelectric point have been obtained [19]. The effect of His-tag at the N-terminus of the amino acid sequence on the properties of PseFDH and $NADP^+$-specific mutants was studied by

**Abbreviations**: FDH – formate dehydrogenase; PseFDH SM4S M2, PseFDH SM4S M3, and PseFDH SM4A M3 are mutant PseFDH SM4S with double S131A/E170D, triple S131A/E170D/S160A, and quadruple S131A/E170D/S160A/S145A substitutions, respectively.