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ORIGINAL ARTICLE

Engineering the Active Site of Formate Dehydrogenase from *Staphylococcus Aureus*: Introduction to the Structure of the Additional Loop and Histidine Residues

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Abstract—NAD^+-dependent formate dehydrogenase (EC 1.2.1.2, FDH) from pathogenic bacterium *Staphylococcus aureus* (SauFDH) differs significantly from other FDHs both in terms of primary structure and catalytic properties. A distinctive feature of SauFDH is the highest (about 2.5–3 times) specific activity compared to other formate dehydrogenases. At the same time, SauFDH has high Michaelis constants for both substrates. Based on the analysis of three-dimensional structures and the alignment of amino acid sequences, replacements promising in terms of changing catalytic parameters were selected. The replacement of I220H resulted in an increase in K_M^{NAD+}; the value of k_{cat} has not changed. When T250H is replaced, an increase in K_M^{NAD+} is observed, k_{cat} decreases from 20 to 13 s^{-1}. The replacement of K368H led to a slight increase in K_M^{NAD+}, k_{cat} decreased from 20 s^{-1} to 6 s^{-1}. The introduction of TGA and AGA additional inserts in α-helix at the C-terminus of the enzyme led to an increase in K_M^{NAD+} and K_M^{HCOO-}. A bigger effect was observed for K_M^{NAD+}—the difference was more than 10 times. For mutant SauFDH with insertions k_{cat} significantly reduced to 4 s^{-1}. Similar results were observed for mutants with multipoint replacements. Thus, the C-terminal sequence has been shown to play an important role in the catalysis of SauFDH.

Keywords: NAD^+-dependent formate dehydrogenase, site-directed mutagenesis, *Staphylococcus aureus*, structure, C-terminus, modeling

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INTRODUCTION

$NAD(P)^+$-dependent formate dehydrogenase (EC 1.2.1.2, FDH) catalyzes the oxidation of formate ion to carbon dioxide with conjugate reduction of $NAD(P)^+$ to NAD(P)H. FDH consists of two identical subunits, each has a coenzyme-binding (residues from 147 to 333 for formate dehydrogenase from *Pseudomonas* sp. 101 (PseFDH)) and a catalytic (amino acid residues 1-146 and 334-400) domains. Each subunit contains an active site, which is located inside of the protein globule.

FDH genes have been found in bacteria, yeasts, fungi, and plants, both higher and lower [1–3]. It has been shown that, under stress conditions, the quantity of the mRNA encoding FDH increases sharply. Accordingly, this enzyme plays an important role in cell metabolism. Particularly, this applies to pathogenic bacteria when they form biofilms [4].

At present, the use of FDH as a catalyst for the regeneration of nicotinamide cofactors NAD(P)H in enzymatic systems has become traditional [5–8]. This is explained by FDH favorably stands out against the background of other enzymes with a similar function due to the low cost of the substrate ($HCOO^-$) and the volatility of the reaction product. However, according

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