



Alteration of coenzyme specificity of malate dehydrogenase from *Streptomyces coelicolor* A3(2) by site-directed mutagenesis

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ABSTRACT. We describe here for the first time the alteration of coenzyme specificity of malate dehydrogenase (MDH) from *Streptomyces coelicolor* A3(2) (*ScMDH*). In the present study, we replaced four amino acid residues in the Rossmann fold ($\beta\text{B}-\alpha\text{C}$) region of NADH-dependent *ScMDH* by site-directed mutagenesis with those of NADPH-dependent MDH (Glu42Gly, Ile43Ser, Pro45Arg, and Ala46Ser). The coenzyme specificity of the mutant enzyme (*ScMDH*-T4) was examined. Coenzyme specificity of *ScMDH*-T4 was shifted 2231.3-fold toward NADPH using $k_{\text{cat}}/K_{\text{m}}^{\text{coenzyme}}$ as the measurement of coenzyme specificity. Accordingly, the effect of the replacements on coenzyme specificity is discussed. Our work provides further insight into the coenzyme specificity of *ScMDH*.

Key words: Malate dehydrogenase; Coenzyme specificity; *Streptomyces coelicolor*; Site-directed mutagenesis