Studies on enzymatic properties and crystal structure of L-leucine dehydrogenase from a psychrophilic bacterium Sporosarcina psychrophila

赵, 莹
九州大学大学院生物資源環境学府

https://doi.org/10.15017/25176

出版情報：九州大学, 2012, 博士（農学）, 課程博士
バージョン：
権利関係：

氏　　名　：赵　莹

論文題目　：Studies on enzymatic properties and crystal structure of L-leucine dehydrogenase from a psychrophilic bacterium *Sporosarcina psychrophila*
（低温細菌 *Sporosarcina psychrophila* 由来 L-ロイシン脱水素酵素の酵素学的特徴及び結晶構造に関する研究）

区　　分　：甲

論　文　内　容　の　要　旨

L-Leucine dehydrogenase (LeuDH, L-leucine: NAD^+ oxidoreductase, deaminating, EC 1.4.1.9) is an oxidoreductase that catalyzes the reversible deamination of L-leucine to 4-methyl-2-oxopentanoate in presence of NAD^+ as a cofactor. The enzymes from mesophiles and thermophiles have been so far purified, characterized, and utilized for the L-amino acid determination and production, but not yet from psychrophiles.

In this study, LeuDH activity was screened in six psychrophilic bacteria, and the highest levels of enzyme activity were found in *Sporosarcina psychrophila* DSM 3. The enzyme was purified from *S. psychrophila* cells to homogeneity and characterized. The protein had an octameric structure with identical 43-kDa subunits, giving a total molecular mass of about 340 kDa. The enzyme exhibited the highest activity at 50˚C and exhibited one-tenth of that activity even at temperatures as low as 0˚C. The enzyme lost no activity with incubation at temperatures lower than 40˚C for 40 min, but did marked loss of the activity higher than 50˚C, indicating that the psychrophilic enzyme exhibits less thermostability compared with those of mesophilic and thermophilic LeuDHs. The optimum pHs were 11 for deamination of L-leucine and 9 for amination of 4-methyl-2-oxopentanoate. The K_m values for L-leucine and NAD^+ at 20˚C were 0.65 and 0.015 mM, respectively. Comparison of enzymatic characterizations showed that the catalytic properties of *S. psychrophila* LeuDH were similar to those of LeuDHs from mesophilic *Lysinibacillus sphaericus* and thermophuilic *Geobacillus stearothermophilus*, except for its relatively higher activity even at lower temperature like 10˚C. Crystal structural analysis of *S. psychrophila* LeuDH was determined, showing the first success of 3-dimensional structure for psychrophilic LeuDH. According to the structural comparison of the psychrophilic LeuDH with that of the *L. sphaericus* one, only minor alterations such as the reduced hydrophobic interactions and number of hydrogen bonds within subunit and at the interfaces between subunits was found, although the total structure was quite similar to each other.

The results obtained in this thesis showed that as the first LeuDH isolated from a psychrophilic bacterium, the *S. psychrophila* enzyme may be useful for practical applications due to its catalytic efficiency even at low temperatures. In addition, the structural analysis of psychrophilic enzyme may afford us some information about relationships between the function and structure with respect to higher reactivity of psychrophilic enzymes at low temperatures, although much more detailed biochemical and genetic engineering analyses about the relationships are needed.