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Unique substrate specificity of glutamate dehydrogenase from thermoacidophilic archaeon *Saccharolobus solfataricus*

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1 Introduction

Glutamate dehydrogenases (GDH, EC1.4.1.2-4) catalyze the reversible oxidative deamination of L-glutamate to 2-oxoglutarate (2-OGA) and ammonia using $NAD(P)^+$ as a coenzyme. GDHs are widely distributed among organisms from archaea to eukaryotes and are a family of enzymes for which there is abundant information concerning their enzymological properties and the relationship between their structure and function.

Within the genome of the thermoacidophilic archaeon *Saccharolobus solfataricus* P2, four putative GDH genes, *SSO1457*, *SSO1907*, *SSO1930*, and *SSO2044* (amino acid sequence identities: 82-92%), have been identified. As a preliminary experiment, we expressed *SSO1457* from *S. solfataricus* in *Escherichia coli* and examined the catalytic properties of the product (SSO1457). We found that in the presence of NAD^+ the enzyme exhibited much higher activity with L-norvaline than with L-glutamate at temperatures between 55°C and 75°C (Fig. 2a). At a substrate concentration of 50 mM, SSO1457 showed higher activity with L-norvaline than L-glutamate below 70°C, but this was reversed above 80°C. No other GDHs are known to show these reaction behaviors.

In this study, we determined the crystal structures of SSO1457 in complex with NAD^+ and 2-oxovalerate (2-OVA, L-norvaline oxo-analog) (PDB ID: 8kao) and with NAD^+ and 2-OGA (L-glutamate oxo-analog) (PDB ID: 8kar) [1]. We then performed molecular dynamics (MD) simulations to investigate the substrate binding state at 80°C. Our findings may contribute to the elucidation of the unique substrate specificity of SSO1457.

2 Experiment

Data were collected under cryo conditions at the Beamline BL5A at Photon Factory in Japan. The program MOLREP was used for molecular replacement phase determination. The crystal structure of GDH from *Thermotoga maritima* (PDB ID: 1b3b) was served as the search model. Model building was performed using the program Coot, and refinement was carried out using REFMAC5.

3 Results and Discussion

In the 2-OGA/NAD^+-bound SSO1457 structure, the C1-carboxylate group of 2-OGA interacts with the side chains of Lys96 and Asn319. The C2-keto group is recognized by the side chain of Lys108, and the C5-carboxylate group of 2-OGA is fixed by the side chains of Lys72, Arg188, and Ser351 (Fig. 1a). These six residues are completely conserved as Lys116, Asn347, Lys128, Lys92, Arg208, and Ser379, respectively, in the 2-OGA/$NADP^+$-bound *Corynebacterium glutamicum* GDH (PDB ID: 5ijz), and similar interactions between those residues and 2-OGA were observed. The Lys72 and Ser351 residues in SSO1457 respectively correspond to Lys89 and Ser380 in *Clostridium symbiosum* GDH complexed with L-glutamate (PDB ID: 1bgv) and recognize the side chain of the substrate. These observations indicate that the residues involved in the substrate binding of SSO1457 are the same as those in conventional GDHs.

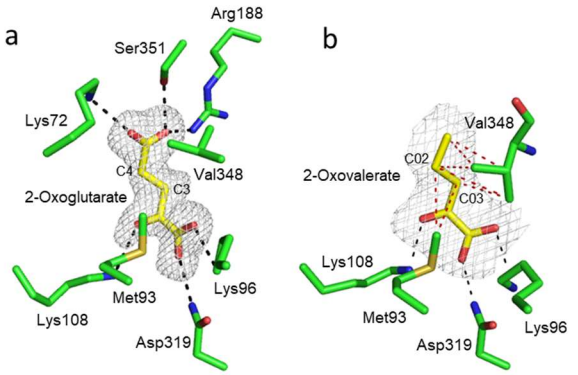

Fig. 1: (a) Close-up of 2-OGA bound to subunit A of SSO1457. Residues surrounding the 2-OGA molecule are shown in green. 2-OGA is shown in yellow. (b) Close-up of 2-OVA bound to subunit C of SSO1457. 2-OVA is shown in yellow. The networks of hydrogen bonds are shown as black dashed lines. The hydrophobic interactions between 2-OVA and Met93/Val348 are shown as red dashed lines. The final Fo-Fc omit electron density map for 2-OGA and 2-OVA was generated using Polder Maps (contoured at 3.5σ).

Comparison of the 2-OVA/NAD^+-bound SSO1457 structure (subunit C) with the 2-OGA/NAD^+-bound SSO1457 structure (subunit A) showed that the positions of the substrate molecule and the surrounding residues in the former are nearly identical to those in the latter (Fig. 1a, b). However, the orientation of the side chain of Val348 differed between the two structures. The Cγ1 and Cγ2 atoms of Val348 in the 2-OVA-bound structure are rotated in an anti-clockwise direction around the Cβ atom by about 112° relative to that of Val348 in the 2-OGA-bound structure. Moreover, the C02 and C03 atoms of 2-OVA are located closer (0.4-0.5 Å) to the side chain of Met93 than the corresponding atoms of 2-OGA (C4 and C3, respectively). Consequently, in the 2-OVA-bound enzyme, the side chains of Met93 and Val348 form hydrophobic interactions with 2-OVA. When we estimated the number of hydrophobic interactions using the WHAT IF web server, we found that Val348 forms five interaction with 2-OVA, while Met93 forms two (Fig. 1b). By contrast, no hydrophobic interactions were observed between 2-OGA and the surrounding residues in the 2-OGA-bound structure.

To reduce hydrophobic interactions between L-norvaline and Val348 or Met93, we constructed V348A and M93A mutants. As shown in Fig. 2b, the V348A mutant still showed greater activity toward L-norvaline than L-glutamate at temperatures around 60-70°C. By contrast, the reaction rates for L-norvaline were markedly reduced in the M93A mutant (Fig. 2c). Notably, the enzyme still exhibited activity toward L-glutamate. These results suggest that the side chain of Met93 is essential for SSO1457 reactivity toward L-norvaline. The hydrophobic interactions between the side chains of L-norvaline and Met93 may contribute to the higher activity toward L-norvaline than L-glutamate.

We next performed MD simulations at 80°C using the NAMD/MOE program. The structure of 2-OGA/NAD^+-bound SSO1457, in which the 2-OGA molecule was replaced by a L-glutamate or L-norvaline molecule, were used as the initial models for the simulation. In the L-glutamate-bound model, hydrogen bonds between substrate and enzyme were maintained during MD simulation, and no major structural movement was observed. (Fig. 3a). On the other hand, in the L-norvaline-bound model, the movement of the L-norvaline side chain was much more pronounced, with loop 1 (residues 72-75) and loop 2 (residues 78-82) of the enzyme moving away from the substrate (Fig. 3b). These observations suggest that the hydrophobic interactions around the L-norvaline side chain weaken at high temperatures and the retention of hydrogen bonds with the side chains of L-glutamate is one of the factors that allow the activity towards L-glutamate to be maintained.

Because the *K*m value for L-norvaline at 85°C (244 mM) is much higher than the *K*m value for L-glutamate (2.9 mM), L-norvaline may not be considered a true substrate for SSO1457. The unique reaction behavior of SSO1457 is an in vitro phenomenon and may not be related to the physiological role. The development of the application aspects of this enzyme, such as the synthesis of L-norvaline, should be the focus of future research.

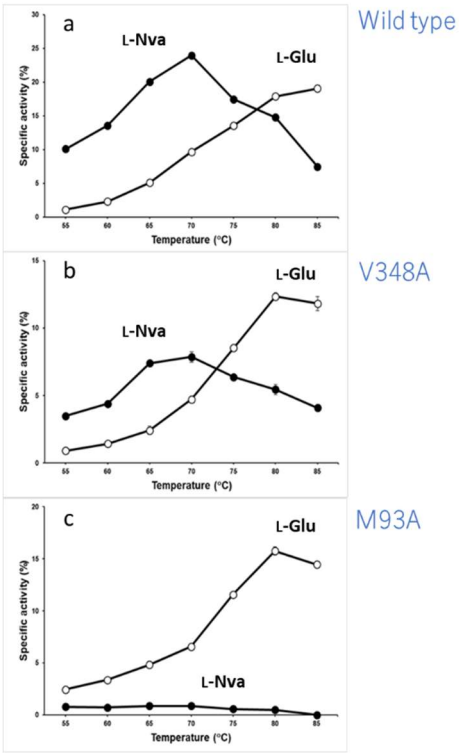

Fig. 2: Effect of temperature on oxidative deamination of L-glutamate (open circles) and L-norvaline (closed circles): wild type (a), V348A (b), and M93A (c).

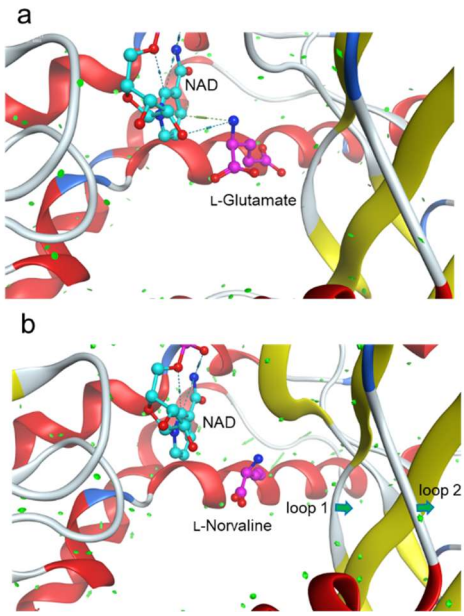

Fig. 3: Molecular dynamics simulation analyses of the L-glutamate-bound model (a) and the L-norvaline-bound model (b). α-Helices and β-sheets are colored in red and yellow, respectively. NAD^+ and substrates are shown in cyan and magenta, respectively. The green arrows indicate the result of principal component analyses of atomic coordinates on trajectory data obtained from MD simulations

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References

[1] I. Okabe *et al*., *Extremophiles*, *in press* (2025).

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