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Crystal structure of the $NADP^+$/tartrate complex of L-serine 3-dehydrogenase from the hyperthermophilic archaeon *Pyrobaculum calidifontis*

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1 Introduction

A gene encoding L-serine dehydrogenase (L-SerDH; EC 1.1.1.276) was identified in the hyperthermophilic archaeon *Pyrobaculum calidifontis* JCM 11548/VA1. The gene was overexpressed in *Escherichia coli*, and its product was purified and characterized. The expressed enzyme appears to be the most thermostable L-SerDH described to date, and no loss of activity was observed by incubation for 30 min at temperatures up to 100°C [1]. The enzyme showed substantial reactivity towards D-serine, in addition to L-serine. The crystal structures of *P. calidifontis* L-SerDH were determined using the molecular replacement method: the structure in complex with $NADP^+$/L-(+)-tartaric acid (substrate analog) at 1.57 Å [2]. The fold of the catalytic domain showed high similarity with that of *Ps. aeruginosa* L-SerDH. However, the active site structure significantly differed between the two enzymes. Based on the structure of the substrate analog, tartrate, L- and D-serine molecules were modeled into the active site and the substrate binding modes were estimated. A structural comparison suggests that the wide cavity at the substrate binding site is likely responsible for the high reactivity of the enzyme toward both L- and D-serine enantiomers. This is the first description of archaeal L-SerDH structures with bound $NADP^+$ and substrate analogue, and it provides new insight into the substrate binding mechanism of L-SerDH.

2 Experiment

Single-wavelength (1.0 Å) data for *P. calidifontis* L-SerDH was collected on the beamline BL-5A at the Photon Factory. The data were processed using HKL2000 and the CCP4 program suite.

3 Results and Discussion

In the electron density map of *P. calidifontis* L-SerDH obtained from our experimental data, we noticed an extra density within the active site cavity and found that a tartrate molecule could be modeled into that density after construction and refinement of the peptide chain. Indeed, the map clearly defined the precise orientation of the tartrate molecule (Fig. 1), and the oxygen atoms of the C1 and C4 carboxylates formed hydrogen bonds with the side chains of Y218 and T121. The oxygen atoms of the C2 and C3 OH groups formed hydrogen bonds with the side chains of Q174, K170, N173, and T121. In addition, the active site lid structure (T206-G213) from another subunit appears to be involved in forming the substrate binding site via hydrophobic interactions. With these interactions, the tartrate molecule is tightly held near the nicotinamide ring of $NADP^+$. Based on the structure of the tartrate molecule, we modeled the L-serine (substrate) molecule into the active site of *P. calidifontis* L-SerDH (Fig. 2A). In our predictive model, two oxygen atoms of the Cα carboxylate formed hydrogen bonds with the side chains of Y218 and the carbonyl group of the nicotinamide ring, respectively. The oxygen atoms of the Cβ OH group formed hydrogen bonds with the side chains of T121, K170, and N173. As described above, *P. calidifontis* L-SerDH exhibits substantial reactivity toward D-serine (65% of the relative activity observed with L-serine). We, therefore, modeled the D-serine (substrate) molecules into the active site of *P. calidifontis* L-SerDH (Fig. 2B).

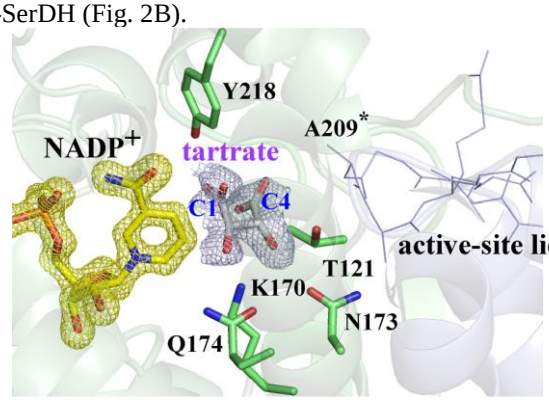

Fig. 1: The $NADP^+$/tartrate-bound structure (5XVH).

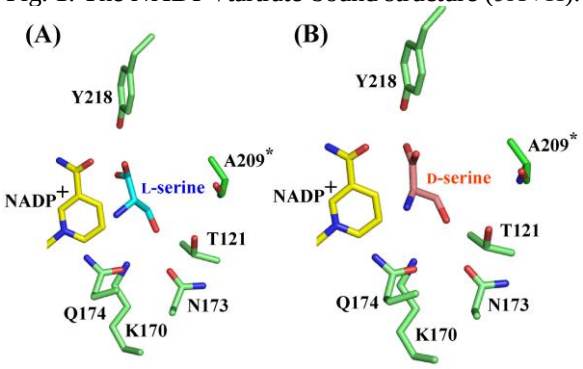

Fig. 2: The $NADP^+$/L-serine-bound (A) and $NADP^+$/D-serine-bound structures (B).

References

[1] K. Yoneda *et al.*, *Acta Crystallographica Section F.* (2013) **F69**, 134-136.

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