Crystal structure of dihydrodipicolinate reductase (*Pa*DHDPR) from *Paenisporosarcina* sp. TG-14: structural basis for NADPH preference as a cofactor

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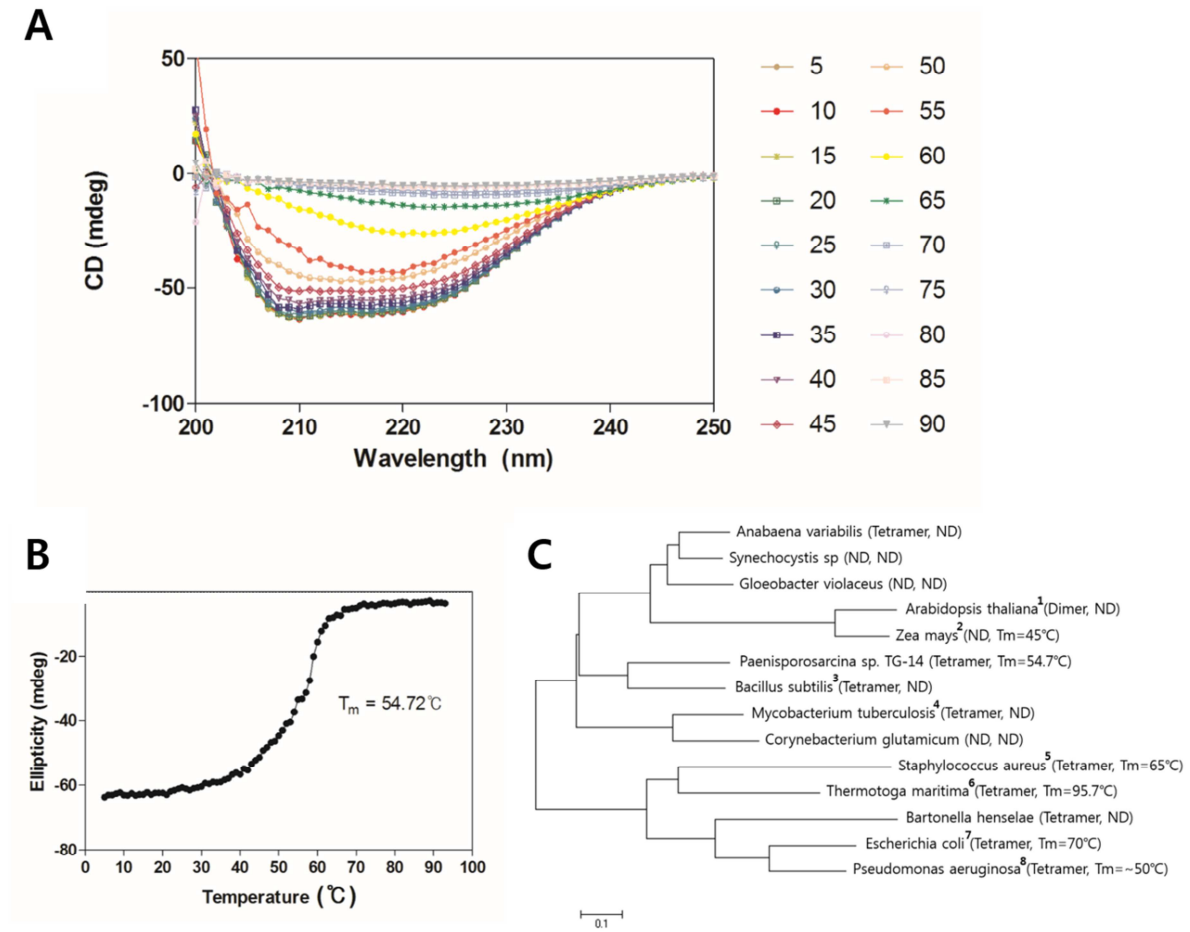
Supplementary Information

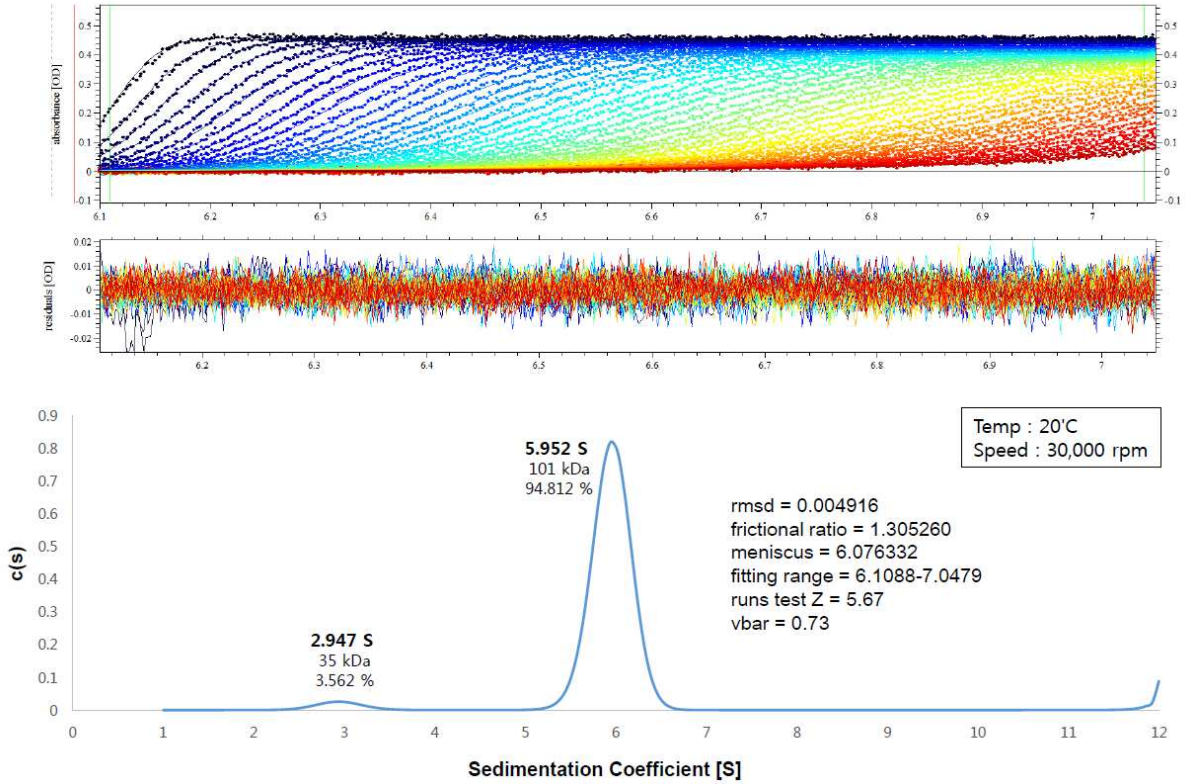
Supplementary Table S1. Kinetic parameters of *Pa*DHDPR.

	NADPH (constant ASA)	**NADH (constant ASA)**	**ASA (constant NADPH)**
K_m (μM)	22 ± 1.4	43 ± 8.9	12 ± 1.6
k_{cat} (s^{-1})	55 ± 1.2	7.1 ± 0.1	52 ± 1.1
k_{cat}/K_m (s^{-1}/μM)	2.5 ± 0.2	0.17 ± 0.04	4.4 ± 0.6

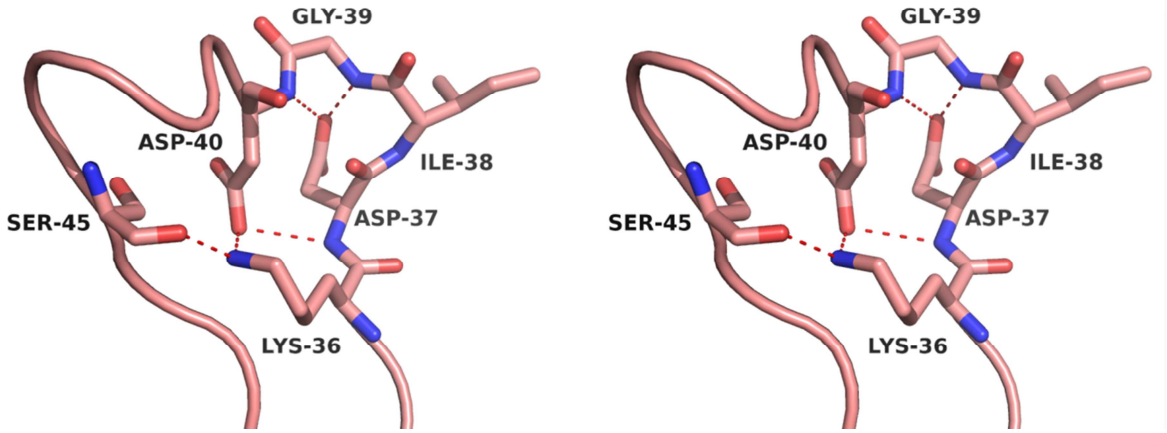
Data are presented as the mean ± standard deviation of three independent experiments.
ASA, L-aspartate-semialdehyde; NADPH, nicotinamide adenine dinucleotide phosphate.



Supplementary Figure. S1. Thermal stability of *Pa*DHDPR monitored by circular dichroism spectroscopy. (a) Typical wavelength scan of *Pa*DHDPR was performed at a given temperature after a 1-min equilibration. (b) Thermal denaturation of *Pa*DHDPR was measured using circular dichroism (Chirascan CD spectropolarimeter; Applied Photophysics, Surrey, UK). *Pa*DHDPR protein solution (1 mg ml^{-1}) in 150 mM NaCl and 20 mM Tris-HCl (pH 8.0) was loaded onto a 0.1-cm path-length cuvette. Changes in ellipticity were monitored at a wavelength of 210 nm by heating the sample to between 5°C and 95°C at intervals of 1°C. The denaturation temperature (T_m) was defined as 54.72C°. (c) The phylogenetic tree based on a multiple sequence alignment of *Pa*DHDPR was drawn using ClustalX2 and MEGA4. The neighbouring-joining method and the bootstrap consensus tree were inferred. The oligomeric state and the melting temperature of DHDPR are given in parentheses.



Supplementary Figure. S2. *Pa*DHDPR analytical ultracentrifugation (AUC). AUC experiments were performed using an XL-A analytical ultracentrifuge (Beckman Coulter, Brea, CA, USA). *Pa*DHDPR protein was diluted in buffer comprising 20 mM Tris-HCl (pH 8.0) and 150 mM NaCl to a final concentration of 0.5 mg/ml. Protein solution and buffer were loaded into the sample and reference sectors of the dual-sector epon centrepiece, respectively. Centrifugation was performed at 45,000 rpm, and sedimentation profile was monitored at 280 nm. Sedimentation-velocity data were analysed with the program SEDFIT[9,10]. AUC experiments on *Pa*DHDPR (residues 1–264; calculated molecular weight: 29 kDa for the polypeptide chain) yielded a mass of 101 kDa (sedimentation coefficient: 5.952 S; frictional ratio: 1.305), indicating that *Pa*DHDPR exists as a tetramer in solution.



Supplementary Figure. S3. Stereo view of internal interactions in the N-terminal long loop (NLL) region. In the apo state, the *Pa*DHDPR NLL region is stabilized by internal interactions. The NZ of Lys36 interacts with the Asp40 side chain and the main-chain carboxyl oxygen atom of Ser45. Asp37 interacts with the main-chain nitrogen atoms of Gly39 and Asp40. The residues involved in internal interactions of the NLL are depicted as slate red sticks.

References

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