CryoEM and SAXS studies on metastable conformations appearing in cofactor-ligand association and catalysis of glutamate dehydrogenase

Enzymes are indispensable biological macromolecules that play critical roles in maintaining the metabolism in living cells. To grasp the fundamental processes of life from physics and chemistry perspectives, understanding the structure and dynamics of enzymes is crucial. Although enzyme structures have traditionally been determined using X-ray crystallography, this method may restrict conformational dynamics due to the molecular contacts necessary to preserve the crystalline arrangements of the enzymes. In contrast, cryoEM may have the potential of visualizing metastable conformations that arise during the enzymatic reaction cycles. In this study, we investigated the metastable conformations appearing in the enzymatic reaction of glutamate dehydrogenase from *Thermococcus profundus* (GDH) using cryoEM (EM01CT: CRYO ARM 300 (K3)) and small-angle X-ray scattering (SAXS) performed at SPring-8 **BL38B1** [1].

GDH catalyzes the deamination converting glutamate to 2-oxoglutarate and ammonia in the presence of cofactor, nicotinamide dinucleotide phosphate (NADP) (Fig. 1(a)). The reaction kinetics of GDH is described by the ordered bi scheme [1]. GDH is composed of six identical subunits, each with a molecular weight of 46k, and each subunit folds into a cofactor-binding domain (NAD domain) and a hexamer-forming domain (core-domain) [2]. Between the two domains, a large active-site cleft is situated. In the unliganded state, the NAD domain exhibits spontaneous hinge and shear motions controlled predominantly by the association and dissociation of several hydration water molecules located at the depth of the active-site cleft [2-4].

In this study, we focused on the metastable conformations that emerge during the enzymatic reaction of GDH [1]. For cryoEM observation for the initial stage, a GDH-NADP-glutamate solution at 278 K was flash-frozen for 15 s after mixing. For the steady stage, a GDH-NADP-glutamate solution at 293 K was flash-frozen at 3600 s after mixing. For each stage, the consensus map of the GDH hexamer was reconstructed at a resolution range of 2.3–2.2 Å, assuming D3 symmetry in the hexamer (Fig. 1(b)). The consensus maps of both stages displayed compaction from the hexamer structure in the unliganded state. Compaction in the steady stage was confirmed by comparing the SAXS data obtained at BL38B1 with that in the unliganded state (Fig. 1(c)). The radius of gyration of GDH in the steady stage was 42.2 ± 0.1 Å, while that in the unliganded state was 43.2 ± 0.1 Å. The NAD domain regions of the consensus map exhibited disorder, particularly at the tip, suggesting that the NAD domain map was in an ensemble of different metastable conformations.

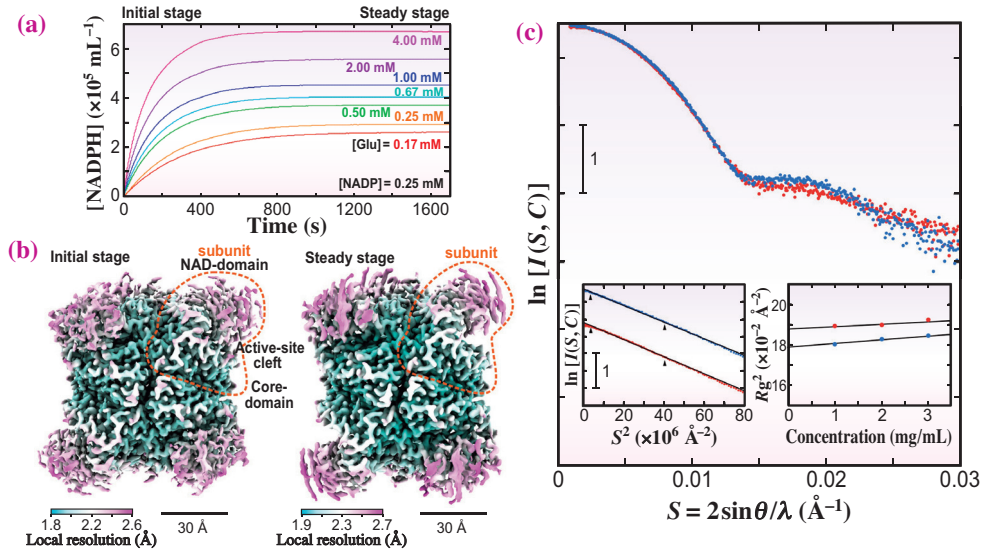

Fig. 1. **(a)** Enzymatic reaction of GDH for 0.17–4.00 mM glutamate in the presence of NADP. The reaction monitored by the concentration of the product NADPH is divided into the initial and steady stages. **(b)** Potential maps of GDH hexamer in initial (left) and steady (right) stages. The maps are shown using the coloring scheme on the local resolution shown at the bottom left in each panel. **(c)** SAXS profiles of GDH (2 mg·mL^{-1}) in the absence (red dots) and in the steady stage (blue dots). The left inset panel is the Guinier plot for SAXS profiles of GDH (1 mg·mL^{-1}). For clarity, the plot for the steady stage was appropriately shifted. The right is the concentration dependencies of the square of the radii of gyration ($Rg^2(C)$).

To separate the metastable conformations, we applied the focused classification protocol to the NAD domain, and revealed four and seven metastable conformations in the initial (Fig. 2) and steady stages, respectively. In each metastable state, a map, interpretable as a cofactor molecule, appeared in the NAD domain. The number of cofactor molecules associated with GDH was consistent with the estimation from the enzyme kinetic analysis for the initial stage.

Among the metastable conformations, the conformations designated as “reaction” had a completely closed active-site cleft and were separated into two states, pre- and post- reaction, with respect to the structure in the reaction pocket. In the pocket of the pre-reaction state, an isolated map assignable to one hydration water molecule used in the deamination reaction appeared near the tip of Lys105. In the post-reaction structure, a map of an ammonium ion appeared near the mainchain of Asn344.

In two metastable conformations, labeled “half-open” and “pre-complex”, respectively, the cofactor maps displayed disorder in the nicotinamide region. We assigned the cofactor molecule NADP based on the differences in the conformational dynamics of NADP and NADPH in solution. As the two conformations had potential maps assignable to glutamate in the reaction pocket, they were categorized as enzyme-cofactor-substrate complexes. The metastable conformation named “pre-open” had a cofactor map that could be fully modeled as NADPH and was assigned as the GDH-NADPH complex after the reaction products were released.

The four metastable conformations were mapped to the ordered bi reaction scheme as illustrated in Fig. 2. The present study suggests that the Michaelis complex, i.e., the GDH-NADP-glutamate complex, in the enzymatic kinetic theory is an ensemble of several metastable conformations.

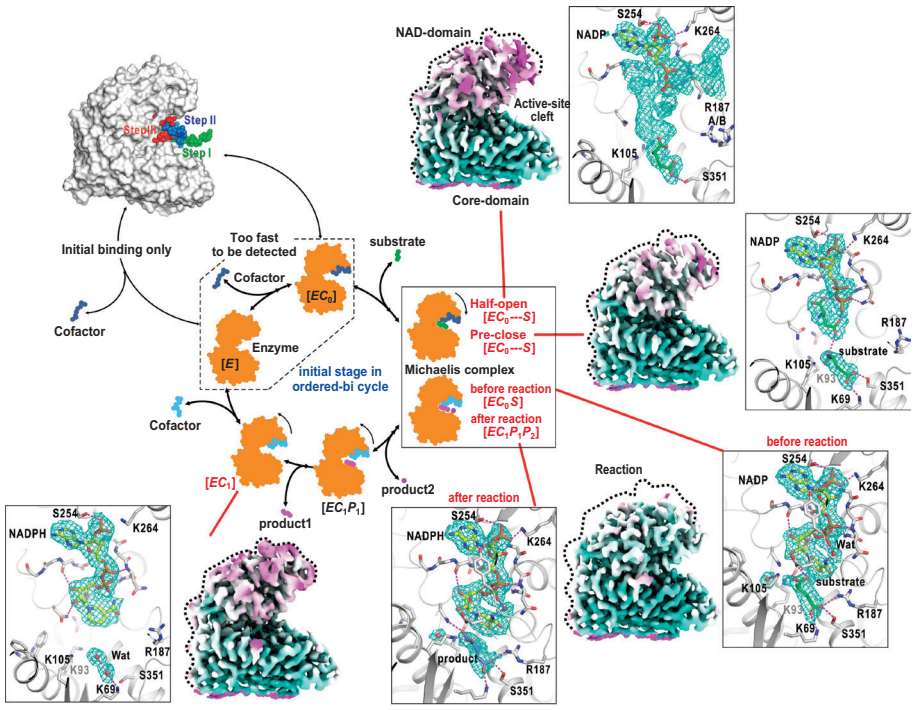

Fig. 2. Structurally identified four metastable conformations of GDH in the initial stage are mapped to kinetic states in the ordered bi reaction cycle. Three of the four metastable conformations are classified into the Michaelis complex from the viewpoint of enzyme kinetic theory. Of these three, the conformations with completely closed active-site clefts are assigned as the pre- and post-reaction states. The other two still had an open cleft and were likely associated with the substrate molecule. The fourth conformation was assigned as the complex of GDH and NADPH. The previous cryoEM study on the GDH-NADP complex suggested that the first NADP binding would occur via a ligand-binding pathway different from that of the ordered bi-cycle. [5]

Taiki Wakabayashi[a,b], Mao Oide[a,b,c,d] and Masayoshi Nakasako[a,b,*]

[a] Department of Physics, Keio University
[b] RIKEN SPring-8 Center
[c] PRESTO, Japan Science and Technology Agency
[d] Protein Research Institute, Osaka University

*Email: nakasako@phys.keio.ac.jp

References

[1] *T. Wakabayashi, M. Oide, M. Nakasako: Sci. Rep.* ***14*** *(2024) 11165.*
[2] M. Nakasako *et al.*: Biochemistry **40** (2001) 3069.
[3] T. Oroguchi and M. Nakasako: Sci. Rep. **6** (2016) 26302.
[4] M. Oide *et al.*: FEBS J. **287** (2020) 3472.
[5] T. Wakabayashi *et al.*: FEBS J. **290** (2023) 5514.