Crystal Structure of P-protein of Glycine Cleavage System from HB8

In most living organisms, the glycine cleavage system (GCS) plays a crucial role in the degradation of glycine. The GCS is a multienzyme complex composed of four different proteins (P, H, T and L) and catalyzes the oxidative cleavage of glycine in a multistep reaction (Fig. 1) [1]. In humans, a mutation in GCS genes can lead to a dramatic accumulation of glycine in blood, resulting in a severe neurological disease termed nonketotic hyperglycinemia (NKH) [2]. Accordingly, the three-dimensional structures of the GCS proteins at the atomic level are required to ultimately understand the molecular basis of NKH. From 1991 to 2004, the structures of H-, T- and L-proteins were elucidated, but only the structure of the P-protein was not reported. The P-protein structure is especially important, because more than 80% of NKH patients have a specific defect in the P-protein [2]. In this study [3], we have determined for the first time the crystal structure of the P-protein.

The P-protein is a pyridoxal 5'-phosphate (PLP)-dependent enzyme (PLP-enzyme) and catalyzes the first step of the GCS reaction (Fig. 1). The P-protein of *Thermus thermophilus* (*Tth*) HB8 forms an $\alpha_2\beta_2$ tetramer with a total molecular mass of 200 kDa. The α- and β-subunits of the *Tth* P-protein were coexpressed in *Escherichia coli*, purified and crystallized as a stable complex [4]. X-ray diffraction data were collected at the RIKEN Structural Biology beamlines **BL44B2** and **BL45XU**. The crystal structure of the P-protein in complex with PLP was determined at 2.1 Å resolution by single isomorphous replacement with anomalous scattering (Fig. 2(a)).

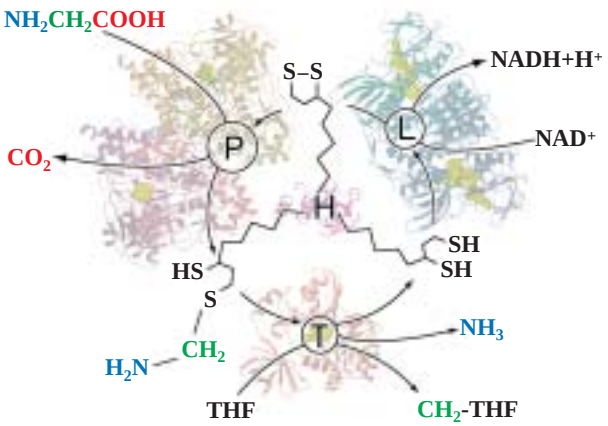

Fig. 1. Multistep reaction catalyzed by GCS.

The P-protein belongs to fold-type I of PLP-enzymes as determined on the basis of sequence similarities [5]. All other fold-type I enzymes with known structures are intimate α_2 dimers, each with an internal twofold axis, or their loose multiples (α_4, α_6 and α_{12}), where an α_2-type active dimer has two active sites at the α-α interface (corresponding to PLP molecules shown in Fig. 2(c,d)). In contrast, two intimate αβ dimers in the P-protein are related by a twofold axis to form an $(\alpha\beta)_2$ tetramer, in which each αβ-type active dimer has only one active site at the α-β interface (Fig. 2(a,b)). The α- and β-subunits show 24% sequence identity and have similar structures; therefore, the αβ dimer appears to have an approximate twofold axis (Fig. 2(b)) and to mimic $\alpha_2$dimers such as those seen in glutamate decarboxylase (Fig. 2(d)). This suggests that the αβ-type active dimer of the P-protein arose by gene duplication of a homodimeric ancestor, after which the ancestral P-protein has structurally diverged such that the protein has been specifically adapted for use as a multienzyme complex component, even though this involved the loss of one active site.

We also analyzed the crystal structures of P-proteins without PLP (i.e. apoenzyme) and in complex with PLP and an inhibitor both at 2.4 Å resolution. These structures allow us to identify functionally important residues involved in the recognition of the cofactor PLP and substrates (glycine and H-protein), and shows that most of these residues are well conserved among all living organisms. This suggests that the catalytic mechanisms should be essentially the same for both the *Tth* and human P-proteins. Shown in Fig. 3 are NKH mutations mapped on a model structure of the human P-protein built on the basis of the *Tth* P-protein. To date, eleven point mutations have been identified in NKH patients. For example, the P329T mutation causes this disease. Pro329 is fully conserved and is involved in the conformational changes upon the binding of PLP. This suggests that P329T affects the binding of the cofactor PLP and thereby leading to the loss of enzymatic activity. Likewise, for most of the other

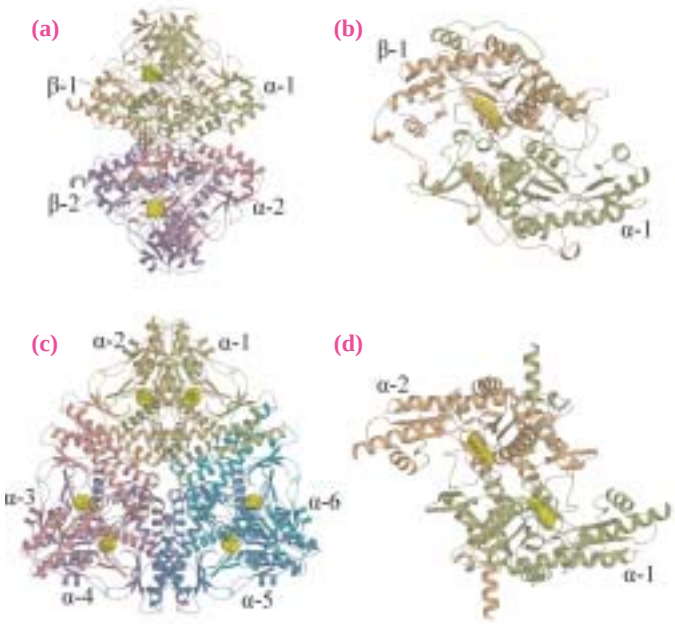

Fig. 2. Crystal structures of $(\alpha\beta)_2$ tetramer **(a)** and $\alpha\beta$ dimer **(b)** of P-protein, and (α_2) 3 hexamer **(c)** and α_2 dimer **(d)** of glutamate decarboxylase. PLP molecules are shown as yellow spheres.

NKH mutations, we have confirmed that the model structure provides a molecular basis for understanding how the mutations lead to the loss of enzymatic activity resulting in the disease [3].

In conclusion, our work on the *Tth* P-protein provides a crystal structure of the final GCS component with an unknown structure. The structural information greatly aids in understanding the molecular pathology of NKH and will be useful in interpreting other NKH mutations that may be identified in the future. Furthermore, it provides new insights into the molecular evolution of P-proteins, a structural basis for understanding catalytic mechanisms, and the architecture of the multienzyme complex, and should stimulate further structure/function studies.

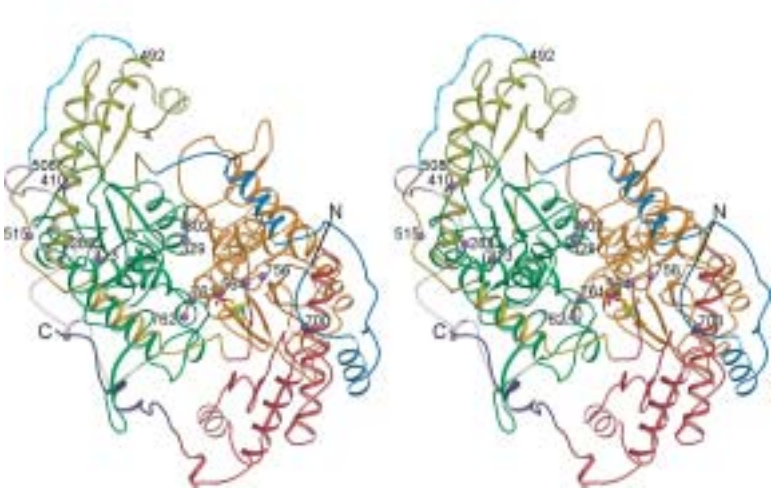

Fig. 3. NKH mutations mapped on a stereo-drawing of model structure of human P-protein.

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