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High-resolution X-ray crystal structure of bovine H-protein at 0.88 Å resolution

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Introduction

Recently, the developments of X-ray crystallography enabled to determine many protein structures and pushed up the resolution limit and, the quality of protein structures. In high-resolution and well-refined structures, we can visualize multiple conformations of main and/or side chains and hydrogen atoms, accurate solvent structures, and determine anisotropic temperature factors. However, examples of high-resolution X-ray crystallography are still not numerous: only ~0.2% structures of the total are beyond 0.9 Å resolution in Protein Data Bank. In this study, bovine H-protein was used as a model protein for high-resolution X-ray crystallography. H-protein plays a central role in glycine cleavage system.

Methods

Crystallization was carried out by the hanging-drop vapour diffusion method and the micro-seeding technique.

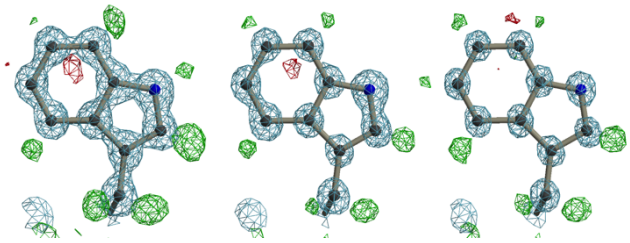
Data collections were performed using synchrotron radiation from Photon Factory beamline BL-5A equipped with an ADSC Quantum315 CCD detector. Three data sets, corresponding to high-, medium- and low-resolution reflections, were collected. To collect the low-resolution data completely, the front-beam stop was removed in order to measure the intensities of the lowest-resolution reflections. All data sets were integrated, scaled and merged using the program *HKL-2000*.

The initial structure of H-protein was determined by molecular replacement using the program *Molrep* using the pea H-protein structure (PDB ID:1hpc) as a search model. The structural refinement was carried out using the program *SHELXL*.

Results and Discussion

We determined the X-ray crystal structure of bovine H-protein at 0.88 Å resolution. Bovine H-protein mainly consists of two antiparallel β-sheets, and helices at the C-terminus joined to the main domain by a flexible linker. Multiple conformations were modeled for 29 residues and ~40% of hydrogen atoms were visualized.

In the measurement of high-resolution X-ray diffraction intensities, several experiments are needed to overcome the limitation of hardware. In this study, data collections for high-resolution diffraction data were carried out in three steps. The combination of these data sets (high-, medium- and low-resolution data set) made a difference in the visualization of hydrogen atoms (Figure 1), and the importance of low-resolution data was confirmed from the experimental data (Figure 2).



(a) (b) (c)

Figure 1. σ_A-Weighted $2F_o$ - F_c (blue) and σ_A-weighted F_o - F_c (positive, green; negative, red) electron-density maps. All figures show the map around residue Trp11. The σ_A-weighted $2F_o$ - F_c (blue) maps are contoured at 1.00 e Å^{-3}. The σ_A-weighted F_o - F_c maps of (*a*) High+Medium+Low-resolution data set, (*b*) High+Medium-resolution data set, (*c*) High-resolution data set are contoured at ±0.15 e Å^{-3}.

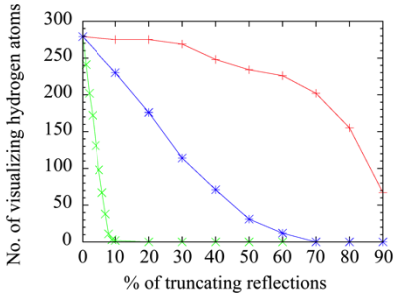


Figure 2. Truncation of reflections. Red and green curves show the truncation of high- and low-resolution reflections, respectively. The blue curve shows random truncation.

Reference

A. Higashiura et al., Acta Cryst D66, 698 (2010).

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