Elucidation of the regulatory mechanism based on the 3-D structures of rat GTP-CH-I and GFRP

Kengo OKADA1, Nobuo MAITA1, Kazuyuki HATAKEYAMA2, Toshio HAKOSHIMA*1
1Nara Institute of Science and Technology, Ikoma, Nara 630-0101, Japan
2Department of Surgery, University of Pittsburgh, USA

Introduction

In mammals, GTP cyclohydrolase I (GTP-CH-I; E.C. 3.5.4.16; 230 residues per monomer) is the first and rate-limiting enzyme in the biosynthesis of 6R-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4) from GTP. BH4 is an essential cofactor for nitric oxide synthase and also for three enzymes, phenylalanine hydroxylase, tyrosine hydroxylase and tryptophan hydroxylase, which play key roles in producing neurotransmitters such as catecholamines from phenylalanine and serotonin from tryptophan. Therefore, the end product BH4 (and also BH2) of the pterin pathway and the starting substrate phenylalanine of the catecolamine pathway are allosteric effectors for GTP-CH-I. Surprisingly, these effectors have no effect on the GTP-CH-I activity without its feedback regulatory protein (GFRP; 83 residues per monomer). Moreover, in the presence of these effectors GFRP is unable to bind GTP-CH-I. Recent studies using gel filtration suggest that free GFRP forms a pentamer and two GFRP pentamers bind the GTP-CH-I decamer, thereby assembling a stimulatory (GTP-CH-I)10-(GFRP)10 complex. To understand the mechanisms by which GFRP regulates GTP-CH-I, we have crystallized the stimulatory ternary complex. The monomeric GTP-CH-I and GFRP have molecular masses of about 257 kDa and 10.0 kDa, respectively. The molecular mass of a single complex is thus approximatively 360 kDa.

Crystallization and data collection

To form the stimulatory complex, GTP-CH-I and GFRP were mixed in a buffer solution containing 50 mM Tris-HCl pH 7.5, 1 mM L-phenylalanine, 100 mM KCl, 1 mM EDTA and 1 mM DTT at final concentrations of 15 μM GTP-CH-I and 15 μM GFRP. The mixture was incubated at room temperature for 20 min and was then concentrated to 15 mg ml⁻¹ by ultrafiltration using Microcon. The sample solution was stored at 193 K.

Crytallization screening was carried out by the hanging-drop vapour-diffusion method using conventional crystallization screening solutions at 288 K. A native data set was collected from one crystal at 100 K on the beamline BL-6A of the Photon Factory using Fuji imaging plates by the rotation method with 2.0° oscillations. Diffraction data were processed and reduced with DENZO and SCALEPACK. The crystals were found to belong to the monoclinic space group P2₁ with unit cell parameters a = 123.3, b = 111.4, c = 125.8 Å, β = 97.69°. Assuming the presence of one complex in the asymmetric unit, the calculated value of crystal volume per protein mass (Vₚ) is 2.4 Å³ Da⁻¹. This value corresponds to a solvent content of approximately 49%. The intensity data collection on the beamline BL-6A gave a set of intensity data at 3.0 Å resolution. The total number of measured reflections was 334,440 and yielded 60,700 unique reflections. The resulting data gave an R_merge of 7.1% with a completeness of 89.9% (75.2% for the outer shell, 3.1-3.0 Å). The mean I/σ(I) ratio was 10.1 (2.82 for the outer shell). The overall redundancy of reflections was 5.5 and the crystal mosaicity was 0.41°. Self-rotation function analyses of the data revealed a strong peak (51.6% height of the origin peak) representing a non-crystallographic fivefold axis. A close inspection of the section k=180° of the self-rotation function revealed a diffuse peaks between each pair of strong peaks. These peaks may suggest that the fivefold axis. These results indicate that the asymmetric unit contains decamers of GTP-CH-I and GFRP with pseudo-52 point-group symmetry. Sturcture analysis by combination of multiple isomorphous replacement and molecular replacement using E. coli GTP-CH-I are in progress.

* hakosima@bs.aist-nara.ac.jp