Biological Science

5A, 6A, 18B, NW12/2004G353

Purification, crystallization and preliminary X-ray diffraction analysis of *Methanococcus jannaschii* TATA box-binding protein (TBP)

Naruhiko ADACHI[1,2,3], Ryo NATSUME[3], Miki SENDA[3], Shinsuke MUTO[1,2], Toshiya SENDA[4], Masami HORIKOSHI*[1,2]
[1]Lab. of Dev. Biol., IMCB, The Univ. of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan
[2]Horikoshi Gene Selector Project, ERATO, JST, Tsukuba, Ibaraki 300-2635, Japan
[3]JBIRC, JBIC, Koto-ku, Tokyo 135-0064, Japan
[4]BIRC, AIST, Koto-ku, Tokyo 135-0064, Japan

Introduction

TATA box-binding protein (TBP) plays a central role in gene expression through the interactions with RNA polymerase and gene-specific transcription factors. As TBP is one of the common components of eukaryotic and archaeal transcription initiation, the investigation of the structural and functional relationship of TBP in more detail is the most attractive strategy to understand the unity and diversity of the mechanism of transcription initiation among eukaryotes and archaea.

The tertiary structure of the core region of eukaryotic TBP showed that TBP has a highly symmetric α/β structure $(\beta\alpha\beta_4\alpha)_2$ which contains a unique DNA binding fold. That of archaeal TBP revealed that the folds of archaeal TBP are almost the same as those of eukaryotic TBP, while that surface properties are quite different from eukaryotic ones. The surface charge of eukaryotic TBP is strongly basic whereas archaeal TBP are comparatively neutral, implying that archaeal TBP have distinct interacting factors from eukaryotic TBP.

Archaeal TBP can be divided into two groups as group I and II, and only the members of group I are structurally known [1]. Although the tertiary structure of group II archaeal TBP has not been solved, it is likely that group II archaeal TBP have different surface properties and interacting factors from group I archaeal TBP. In fact, one of the group II archaeal TBP (*M. jannaschii* TBP) shows a weak TATA box-binding activity, unlike eukaryotic and group I archaeal TBP. To analyze the conservation and diversification of the mechanism of actions of TBP at the atomic level, we have initiated structural and functional studies of *M. jannaschii* TBP. Here we report the purification, crystallization and preliminary crystallographic analysis of *M. jannaschii* TBP.

Methods

M. jannaschii TBP was purified as described earlier [1]. Crystals were grown within one month in three conditions containing PEG 400, PEG 4000, and PEG MME 2000. Further screenings for finding optimal conditions for crystal growth were accomplished by varying the pH, precipitant concentration, and volume of solution. Finally, the best large crystals were obtained at 293K in a drop containing 1.5 µl of 20 mg ml^{-1} protein solution (20 mM Tris-HCl (pH 7.9 at 277K), 10 % glycerol, 100 mM NaCl, 10 mM 2-mercaptoethanol), and 1.5 µl of reservoir solution (0.2 M ammonium sulfate, 0.1 M sodium acetate buffer pH 4.6, and 30 % PEG MME 2000). The data were collected at 100 K from a single crystal using the ADSC Quantum 315 CCD detector on the beamlines BL-5 at the Photon Factory, Tsukuba, Japan. The diffraction data were processed and scaled using the programs MOSFLM and SCALA, respectively.

Results

Crystals grew to approximate dimensions of 0.2 x 0.1 x 0.03 mm^3 around one month. Examination of diffraction data from *M. jannaschii* TBP crystals revealed that these crystals diffract beyond to 1.9 Å resolution and belong to the space group $P2_1$, with unit-cell parameters a = 53.2 Å, b = 55.5 Å, c = 123.4 Å, β = 91.0°. A value for the Matthews coefficient of 2.1 and a solvent content of 40.3 % were obtained assuming four molecules in the asymmetric unit. It was confirmed by a self-rotation map which was calculated with the program POLARRFN in the CCP4 program suite. The self-rotation map suggests that there are two non-crystallographic two-fold axes. Molecular replacement (MR) was performed with the program *MOLREP*. Further crystallographic refinement is in progress.

Table 1: Data-collection and processing statistics

Space group	$P2_1$
Unit-cell parameters	a = 53.2, b = 55.5, c = 123.4 Å, β = 91.0°
Resolution range	62.00 – 1.90
No. of measured reflections	208543
No. of unique reflections	57094
R_{merge} [#] (%)	3.9 (35.0)[$]
Completeness (%)	99.9 (99.9)[$]
Average I/σ	9.2 (2.1)[$]

[#] $R_{merge} = \Sigma|I(\mathbf{h})-\langle I(\mathbf{h})\rangle|/\Sigma I(\mathbf{h})$
[$] Last shell, 2.00 – 1.90 Å.

References

[1] N. Adachi et al., Acta. Crystallogr. D 60, 2328 (2004).

* horikosh@iam.u-tokyo.ac.jp