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Preliminary X-ray diffraction analysis of the periplasmic region of the *E. coli* Tar and its complexed with aspartate

Takeshi Mise* and Ichiro N. Maruyama
Information Processing Biology Unit, Okinawa Institute of Science and Technology Graduate University, 1919-1, Tancha, Onna-son, Kunigami, Okinawa, 904-0495, Japan

1 Introduction

The cell-surface aspartate receptor, Tar, mediates bacterial chemotaxis toward an attractant, aspartate (Asp), and away from a repellent, Ni^{2+}. How the ligands regulate the activity of Tar during chemotaxis is still unknown. Maruyama *et al.* proposed an alternative model in which the repellents stabilize the second transmembrane α-helix in a different rotational orientation from those of the apo- and Asp-bound forms, through the rotation/twist of the second transmembrane α-helix parallel to the plane of the cytoplasmic membrane [1]. The model also predicts that apo-Tar and Asp-Tar have similar structures. To test this model, the *E. coli* Tar periplasmic domain with and without bound aspartate, Asp-Tar and apo-Tar, respectively, were crystallized. The expression, purification, crystallization, and preliminary X-ray diffraction studies of Asp-Tar and apo-Tar were reported [2]. Structure determinations of apo-Tar and Asp-Tar are currently in progress. Crystallization and structural analysis of the periplasmic domain with bound Ni^{2+} is also advancing.

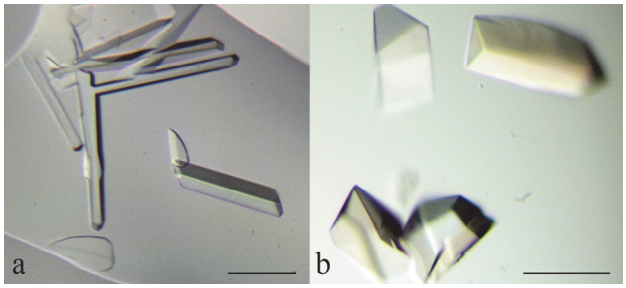

Fig. 1: Crystals of apo-Tar (a) and Asp-Tar (b). Scale bar, 100 μm.

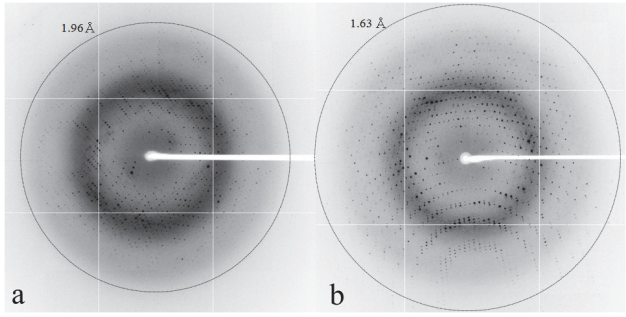

Fig. 2: X-ray diffraction images from crystals of apo-Tar (a) and Asp-Tar (b). Resolution circles are shown with values.

2 Experiment

The procedures of preparation, purification, crystallization, and X-ray diffraction analysis for the periplasmic domain of the aspartate receptor have been reported elsewhere [2].

Table1 Data collection and processing statistics. Values in parentheses are for the outermost resolution shell.

Data collection	**apo-Tar**	**Asp-Tar**
X-ray source	BL5A, PF	BL5A, PF
Detector	ADSC Q315	ADSC Q315
Wavelength (Å)	1.000	1.000
Beam size (μm)	Diameter, 50	Diameter, 50
Camera distance (mm)	204.2	175.8
Oscillation angle (°)	0.5	0.75
Oscillation range (°)	180	180
Exposure time (s)	2.0	2.0
Space group	$P\,2_12_12_1$	$C2$
Unit cell (Å)	$a = 60.29$ $b = 73.31$ $c = 79.14$	$a = 81.71$ $b = 59.67$ $c = 79.35$
Unit cell (°)	$\alpha = 90.00$ $\beta = 90.00$ $\gamma = 90.00$	$\alpha = 90.00$ $\beta = 94.85$ $\gamma = 90.00$
Molecules in ASU	2	2
V_M ($Å^3\ Da^{-1}$)	1.96	2.17
Solvent content (%)	37.43	43.23
Mosaicity (°)	0.53	0.63
Resolution range (Å)	1.96-60.29 (1.96-2.07)	1.63-35.01 (1.63-1.72)
Measured reflections	178646 (26333)	167017 (24143)
Unique reflections	25847 (3706)	47438 (6869)
Completeness (%)	99.9 (100.0)	99.8 (99.9)
R_{merge} (%)	0.063 (0.287)	0.065 (0.291)
Multiplicity	6.9 (7.1)	3.5(3.5)
Mean $I/\sigma(I)$	18.2(6.7)	10.8 (3.5)

3 Results and Discussion

The expression plasmid encodes the entire periplasmic domain of Tar from Gly26 to Gln193, which is the longest of the Tar periplasmic domains that have been crystallized so far. It took a few months to get crystals of apo-Tar (Fig. 1a). On the other hand, many crystal nuclei of Asp-Tar appeared within a week (Fig. 1b). Crystals of apo-Tar and Asp-Tar diffracted to 1.96 Å and 1.63 Å resolutions, respectively (Fig. 2 and Table 1), and adopted space groups $P2_12_12_1$ and $C2$, respectively (Table 1). Structure determinations of apo-Tar and Asp-Tar are currently in progress. Furthermore, crystallization and structural analysis of the periplasmic domain with bound Ni^{2+} is advancing, too.

Acknowledgement

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References

[1] Maruyama *et al*., *J. Mol. Biol.* **253**, 530-546 (1995).
[2] Mise *et al*., *Acta Crystallog. Sect. F* (accept).

*takeshi.mise@oist.jp