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Crystal structure of cyclo/maltodextrin-binding protein

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Introduction

Cyclodextrins (CDs) are cyclic α-1,4-glucans, and the central cavity of CDs can host a large number of chemicals by hydrophobic interaction. A thermophilic actinomycete, *Thermoactinomyces vulgaris* R-47, produces two CD-hydrolyzing enzymes, TVA I and TVA II. We have determined the crystal structures of TVA I and TVA II. To find the proteins physiologically related to these enzymes, the flanking regions of the genes were sequenced. A gene homologous to those of the bacterial sugar-binding protein family was found to be located upstream of the TVA II gene, and the affinities of this protein for γ-CD were higher than that for maltose [1]. The results suggested that this protein participates in binding to not only linear maltooligosaccharides but also CDs, and thus was designated cyclo/maltodextrin-binding protein (*Tvu*CMBP). Here we present the crystal structure of *Tvu*CMBP complexed with γ-CD. The structure provides evidence that the architecture of *Tvu*CMBP is well optimized for interacting with the central hydrophobic cavity of γ-CD.

Materials and Methods

Crystals were grown by the hanging drop vapor diffusion method at 20°C. Crystals of *Tvu*CMBP complexed with γ-CD were obtained by mixing 1μl of well solution (25% PEG 6000, 0.1 M MES pH 6.25, 5 mM γ-CD) and 1μl of protein solution (10 mg/ml *Tvu*CMBP). Finding of the heavy-atom sites and determination of the initial phasing of the SeMet data set were carried out using the program Solve. Although the model of the SeMet-substituted *Tvu*CMBP was initially built, a high R_{free} value was yielded after placing all of the residues, water molecules and γ-CD, probably because of the high mosaicity of the MAD data set. Thus, the native data set was used for further refinement. The atomic coordinates and structural factors (code 2DFZ) have been deposited in the Protein Data Bank.

Results and Discussion

The crystal structure of a *Tvu*CMBP complexed with γ-CD was determined at 2.5-Å resolution (Fig. 1) [2]. Like *Escherichia coli* maltodextrin-binding protein (*Eco*MBP) and other bacterial sugar-binding proteins, *Tvu*CMBP consists of two domains, N-domain and C-domain, both of which are composed of a central β-sheet surrounded by α-helices, and the domains are joined by a hinge region containing three segments. γ-CD is located at a cleft formed by the two domains. A common functional conformational change has been reported in this protein family, which involves switching from an open form to a sugar-transporter bindable form, designated a closed form. The *Tvu*CMBP-γ-CD complex structurally resembles the closed form of *Eco*MBP, indicating that *Tvu*CMBP complexed with γ-CD adopts the closed form. Despite having similar folds, the sugar-binding site of the N-domain part of *Tvu*CMBP and other bacterial sugar-binding proteins are strikingly different. In *Tvu*CMBP, the side-chain of Leu59 protrudes from the N-domain part into the sugar-binding cleft and orients toward the central cavity of γ-CD, thus Leu59 appears to play the key role in binding. The cleft of the sugar-binding site of *Tvu*CMBP is also wider than that of *Eco*MBP. These findings suggest that the sugar-binding site of the N-domain part and the wide cleft are critical in determining the specificity of *Tvu*CMBP for γ-CD.

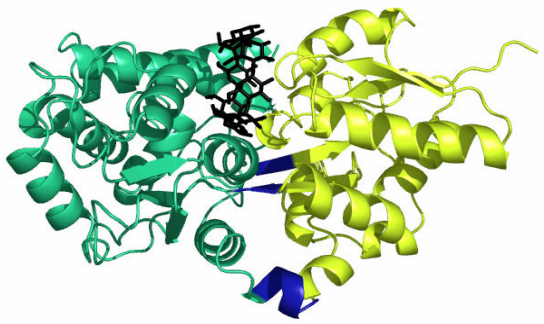

Fig. 1. Structure of *Tvu*CMBP. N- and C-domains, and hinge regions are shown in yellow, green and blue, respectively. The γ-CD molecule is indicated in black.

References

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