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The C2′- and C3′-endo equilibrium for AMP molecules bound in the cystathionine-beta-synthase domain

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ABSTRACT

The equilibrium between C2′- and C3′-endo conformations of nucleotides in solution, as well as their polymers DNA and RNA, has been well studied in previous work. However, this equilibrium of nucleotides in their binding state remains unclear. We observed two AMP molecules, in C3′- and C2′-endo conformations respectively, simultaneously bound to a cystathionine-beta-synthase (CBS) domain dimer of the magnesium and cobalt efflux protein CorC in the crystallographic study. The C2′-endo AMP molecule assumes the higher sugar pucker energy and one more hydrogen bond with the protein than the C3′-endo molecule does. The balance between the high sugar pucker energy and the low binding energy suggests an equilibrium or switch between C2′- and C3′-endo conformations of the bound nucleotides. Our work challenge the previous hypothesis that the ribose of the bound nucleotides would be locked in a fixed conformation.

1. Introduction

The 5′-*ribo*- and 5′-deoxyribonucleotides assume a variety of conformations, which are mainly determined by the sugar pucker along with the rotations of the base around *N*-glycosidic bond and the C5′-hydroxymethyl group around the C4′-C5′ bond. This intrinsic conformational variety confers the structural variety of DNA and RNA polymers. Specially, the D-ribose and D-deoxyribose rings of all the 5′-nucleotides have an equilibrium between C2′-endo and C3′-endo conformations in solution, which are also termed as S type and N type conformers respectively [1]. The minimum energy conformations of the ribose moiety of 5′-*ribo*- and 5′-deoxyribonucleotides, along with those in RNA and DNA polymers (RNA and DNA), are C3′- and C2′-endo conformations respectively, although most RNA and DNA monomers tend to a predominant C2′-endo conformation in solution [2]. The C2′- and C3′-endo equilibrium or switch are also involved in the switch of B— and A-form DNA (as well as RNA), the process of DNA/RNA binding to their partners [3,4], the repuckering dynamics of excited state RNA [5] and the activation of ribonucleotides in template-directed non-enzymatic primer-extension reactions [2]. Notably, the chemical modification on the sugar moiety of nucleotides that influence the sugar ring pucker was also conformed to influence the biological activities of nucleic acids [6–8], suggesting the key role of the ribose puckers.

This equilibrium has been well studied for DNA/RNA monomers in solution and for DNA/RNA polymers [2–4]; however, that of nucleosides or nucleotides in their binding state are lack of study because NMR assay is not suitable in this situation. It was excepted that a nucleoside or nucleotide binds to their partner proteins or target enzyme with only one predominant conformation since the energy barrier between C3′- and C2′-endo conformations, approximately 4 kcal/mol [8,9], could lead to a disparity between micromolar and nanomolar affinities [10,11] and lock the ribose in a fixed conformation [12].

During the crystallographic study of the cystathionine-beta-

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synthase (CBS) domain of magnesium and cobalt efflux protein CorC (hereafter named as CorC-CBS), we observed two AMP molecules, in C3′- and C2′-endo conformations respectively, simultaneously bound to a CorC-CBS dimer. Our observation elucidates the structural basis that the CBS domain could bind AMP molecules in a predominant either C3′- or C2′-endo conformation or in the equilibrium of both them. Further, we proposed and then confirmed that CBS domains could bind dAMP with a similar affinity to AMP. Our study renew the previous knowledge of CBS domains, lead to more questions about ligand recognition of CBS domain, and suggest that the C2′- and C3′-endo conformation equilibrium or switch could be involved in the recognition of AMP and other adenosyl derivatives by CBS domains.

2. Materials and methods

2.1. Protein expression and purification

The CBS domain of CorC was amplified by PCR and cloned to a pET22b vector using *Nde*I and *Xho*I restriction sites. Recombinant vector was transformed into *E. coli* BL21 (DE3) cells. Transformed cells were cultured at 37 °C in LB medium containing 100 mg/l ampicillin until OD600 reached 0.8. Expression of CBS protein was induced by adding 0.5 mM isoporopyl-D-thiogalactopyranoside (IPTG) and cells were cultured at 16 °C for 20 h. Harvested cells were lysed by sonication in buffer containing 50 mM Tris (pH 8.0), 300 mM NaCl and 10 mM imidazole. The lysate was separated by centrifugation and loaded on a Ni^{2+}-NTA (Qiagen) column for immobilized metal affinity chromatography. The column was washed in buffer containing 20 mM imidazole. Protein was eluted by using 300 mM imidazole. Eluted protein was treated by 2 M urea overnight to remove the originally combined ligand. Treated protein was further purified using a Hitrap-Q column (GE healthcare) with buffer A in 50 mM Tris (pH 8.0) and 2 M urea and buffer B in 50 mM Tris (pH 8.0), 2 M urea and 1 M NaCl. Then the concentrated protein was loaded on a Superdex 75 10/300 size exclusion column (GE healthcare) in 50 mM Tris (pH 8.0) and 100 mM NaCl. Mutants were purified as the same steps.

2.2. Crystal preparation and structure determination

Crystallization was screened using hanging-drop vapor diffusing in 96-well culture plates and was optimized in 24-well culture plates at 22 °C. The CBS protein and AMP were pre-mixed with the final concentration of 10 mg/mL and 5 mM respectively. Mixture were incubated at 4 °C for half an hour before crystallization. Crystal of complex was grown in solution containing 0.1 M Bis-Tris pH5.5, 25% PEG3350 and 0.2 M NaCl. The crystal was soaked quickly in cryoprotectant with 10% glycerol and then flash frozen in liquid nitrogen.

Diffraction data were collected at beamline BL19U1 of Shanghai Synchrotron Radiation Facility. Data were processed in XDS [13] and the structure was determined with Phaser [14] by molecular replacement using 4HG0 as the search model. Phenix [15] and Coot [16] were used for subsequently refinement. The data collection and structure refinement statistics were summarized in Table 1. All structure figures were generated with PyMOL [17].

2.3. Isothermal titration calorimetry (ITC)

Binding affinities between all proteins and different nucleotides were measured using isothermal titration calorimetry. The ITC experiments were carried out by using a ITC-200 microcalorimeter (GE healthcare) at 25 °C, with 0.05 mM proteins in cell and 0.5 mM ligands in syringe. All the proteins and ligands were diluted in buffer containing 20 mM Tris pH 8.0 and 100 mM NaCl. All the samples were thoroughly degassed and centrifuged to remove precipitates. Volumes of 1 μL per injection were used and 2.5 min intervals were set to allow the peak to return to the baseline. ITC data were fitted in a one-site model using Origin 7.0 software.

Table 1
Crystallographic data collection and refinement statistics.

	CorC_CBS-AMP (PDB ID: 5YZ2)
Data collection	
Space group	P 1 2_1 1
Cell dimensions	
a, *b*, *c* (Å)	53.90, 56.29, 54.35
α, β, γ (°)	90.00, 114.73, 90.00
Resolution (Å)	50.00–1.75 (1.80–1.75)[a]
No. reflections	29796 (2139)
R_{merge}	0.047 (0.776)
$I/\sigma I$	21.04 (2.44)
Completeness (%)	99.3 (97.2)
Redundancy	6.5 (6.0)
CC(1/2)	0.999 (0.823)
Refinement	
R_{work}/R_{free}	0.209/0.235
No. atoms	
Total	2367
Protein	2142
Ligand/ion	70
Water	155
B-factors	
Total	40.2
Protein	39.9
Ligand/ion	35.5
Water	46.5
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.759
Ramachandran Plot	
Outliers (%)	0.00
Allowed (%)	1.14
Favored (%)	98.86

[a] Values in parentheses are for the highest resolution shell.

2.4. Surface plasmon resonance (SPR)

Surface plasmon resonance assay was used to determine the binding kinetics of all proteins and the nucleotides. The experiments were performed on a Biacore T100 (GE healthcare). Proteins were labelled with biotin respectively and immobilized onto a streptavidin-coated sensor chip (SA sensor) to about 1100 response units (RU). Different nucleotides were diluted into different concentrations in running buffer (20 mM Tris pH 8.0, 100 mM NaCl and 0.05% Tween20) and injected at a flow rate of 10 μL/min at 16 °C. SPR data were fitted with a steady model or a kinetic model according to the binding characters.

3. Result

3.1. Overall structure and AMP recognition of CorC-CBS

The CorC-CBS protomer contains two tandem copies of CBS motifs: CBS1 and CBS2. Both motifs adopt the typical fold of CBS motif, including a three-stranded β-sheet and two α-helices in the sequence order of β1-α1-β2-β3-α2 along with a flexible linker that preceded the first β-strand. They assembled into a pseudo-2-foled symmetric clamp-like structure, with the preceded linker packing with the *C*-terminal region β3-α2 of the other CBS motif in antiparallel. Two protomers in a crystallographic asymmetric unit were observed to form an elliptical disk-like assembly dimer with