Biochemical and Biophysical Research Communications 477 (2016) 477–482

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Six independent fucose-binding sites in the crystal structure of *Aspergillus oryzae* lectin

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ARTICLE INFO

Article history:
Received 8 June 2016
Accepted 14 June 2016
Available online 16 June 2016

Keywords:
Lectin
Seleno-sugar
Fucose
Affinity
Phase determination
Crystal structure

ABSTRACT

The crystal structure of AOL (a fucose-specific lectin of *Aspergillus oryzae*) has been solved by SAD (single-wavelength anomalous diffraction) and MAD (multi-wavelength anomalous diffraction) phasing of seleno-fucosides. The overall structure is a six-bladed β-propeller similar to that of other fucose-specific lectins. The fucose moieties of the seleno-fucosides are located in six fucose-binding sites. Although the Arg and Glu/Gln residues bound to the fucose moiety are common to all fucose-binding sites, the amino-acid residues involved in fucose binding at each site are not identical. The varying peak heights of the seleniums in the electron density map suggest that each fucose-binding site has a different carbohydrate binding affinity.

1. Introduction

L-Fucosyl residues of glycoproteins on the cell surface play important roles in many biological processes. Altered fucosylation of glycoproteins is associated with serious diseases including cancer, cystic fibrosis, and infection by pathogens. For example, the level of fucosylated α-fetoprotein (AFP) is elevated in liver disease [1,2]. Therefore, in order to diagnose or prevent serious liver diseases, it is necessary to monitor the level of AFP. Fucose-binding lectins from plants and fungi have been used for this purpose [3,4]. Fucose-binding lectins have broad binding specificities in general, and some of them preferentially recognize the α1,6-fucose moiety [5–7]. FleA from *Aspergillus oryzae* (AOL), which was originally identified as a ferrichrysin (Fcy) affinity protein, is also a fucose-specific lectin [8]. Purified AOL promotes hemagglutination of rabbit erythrocytes. Furthermore, hemagglutination inhibition assays revealed that AOL is an L-fucose–specific lectin.

Many three-dimensional structures of fucose-binding lectins have been determined and classified [9]. One major family of the fucose-binding lectins whose structures have been solved is called "fungal fucose-specific lectins", which include lectins from *Aleuria aurantia* (AAL) [10,11], *Aspergillus fumigatus* (AFL) [12], *Ralstonia solanacearum* (RSL) [13], and *Burkholderia ambifaria* (BambL) [14]. Among these, AAL and AFL are from fungi, whereas RSL and BambL are from bacteria. The primary feature of AAL and AFL structure is a six bladed β-propeller. Monomers of bacterial lectins, which consist of two-bladed β-propellers, form trimers. Thus, the final trimeric structure is a six-bladed β-propeller fold, similar to that of the fungal lectins [13,14]. In the case of RSL and BambL, all six fucose-binding sites in the six-bladed β-propeller structure are occupied by fucose moieties. On the other hand, the proteins differ with respect to the numbers of bound carbohydrates: all six sites are occupied in AFL [12], whereas only five [10] or three [11] are occupied in AAL.

Here, we report the crystal structure of a fungal fucose-specific lectin, AOL, in complex with seleno-fucosides. Seleno-

Abbreviations: AOL, fucose-specific lectin from *Aspergillus oryzae*; AAL, fucose-specific lectin from *Aleuria aurantia*; AFL, fucose-specific lectin from *Aspergillus Fumigatus*; α-Se-Fuc, α-seleno-fucoside derivative; β-Se-Fuc, β-seleno-fucoside derivative; MAD, multi-wavelength anomalous diffraction; SAD, single-wavelength anomalous diffraction; RMSD, root mean square deviation.

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http://dx.doi.org/10.1016/j.bbrc.2016.06.069

carbohydrates are useful for phase determination of sugar-binding proteins, and several X-ray crystal structures of sugar-binding proteins in complex with seleno-carbohydrates are determined [12,13,15–17]. In this study, we applied multi-wavelength anomalous diffraction (MAD) and single-wavelength anomalous diffraction (SAD) using seleno-fucosides to solve the structure of AOL, and estimated the fucose-binding affinity at each site from the height of the selenium peak in the electron density map.

2. Materials and methods

2.1. Seleno-fucosides

Seleno-fucosides were synthesized via transacetalization between a selenoacetal and a glycosyl imidate [18]. In this study, reaction between fucosyl imidate [19] and a benzyloxymethyl methyl selenide in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave methyl 3,4-di-*O*-acetyl-6-deoxy-2-*O*-(4-methoxybenzyl)-1-seleno-L-galactopyranoside in 87% yield (α:β = 3.14:1). α- and β-seleno-fucoside derivatives [α-Se-Fuc and β-Se-Fuc, respectively (Fig. 1A),] were prepared by removal of the 4-methoxybenzyl group followed by deacetylation, with good overall yield. Detailed synthetic procedures will be reported elsewhere. The synthesized seleno-fucoside was dissolved in distilled water as a 100 mM stock solution and stored at −80 °C before crystallization.

2.2. Crystallization

Recombinant AOL was purchased from Tokyo Chemical Industry (Product number L0169, 5 mg/mL, in PBS at pH 6.5). After concentration to 7.1–7.8 mg/mL in PBS, the sample was used for crystallization. The AOL solution was mixed with 10 mM seleno-fucoside (α-Se-Fuc or β-Se-Fuc) on ice for 30 min before crystallization. Crystallization screening was performed by a crystallization robot [20]. Crystals of AOL with α-Se-Fuc or β-Se-Fuc were obtained under several conditions. The best crystal of AOL with α-Se-Fuc was obtained at 20 °C in 2% v/v PEG-400, 2.0 M ammonium sulfate, 0.1 M HEPES-Na (pH 7.5). The best crystal of AOL with β-Se-Fuc was obtained at 20 °C in 1.0 M mono-ammonium dihydrogen phosphate, 0.1 M tri-sodium citrate dihydrate (pH 5.6). 20% w/v glycerol was used as a cryoprotectant for collection of both data sets.

2.3. Data collection and structure determination

MAD data for AOL with α-Se-Fuc and SAD data for AOL with β-Se-Fuc were collected at the BL-17A and BL-5A beamlines of the KEK Photon Factory (Tsukuba, Japan), respectively. Before data collection, X-ray absorption fine structure (XAFS) experiments were performed and analyzed using CHOOCH [21] to determine the absorption edge (peak) and inflection point of seleno-fucosides. All data were collected at 100 K and processed using the XDS package [22,23]. Data scaling was performed with Pointless and Aimless of the CCP4 suite [24,25]. The initial selenium sites of α-Se-Fuc in the complex were determined with SHELXD in autoSHARP using the

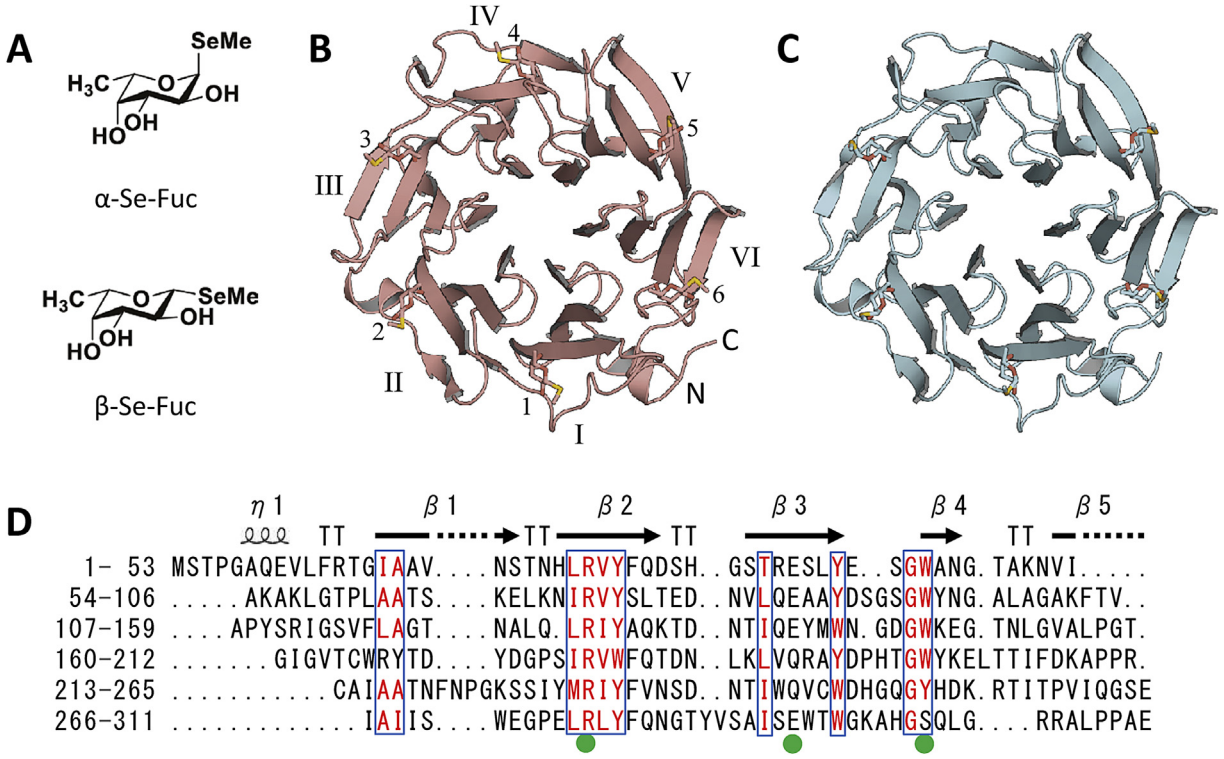

Fig. 1. Overall structure of AOL and seleno-fucosides used in structure determination. (A) Chemical structures of seleno-fucosides used in this study. (B) Overall structure of AOL with β-Se-Fuc is represented as a ribbon model in pink. The seleno-carbohydrates in the fucose-binding sites are represented by stick models, labeled from 1 to 6. N and C refer to the N and C termini of AOL, respectively. The six blades of AOL are numbered from I to VI. (C) The overall structure of AOL containing α-Se-Fuc is represented by a ribbon model in cyan. The orientation of AOL containing α-Se-Fuc is aligned with that of AOL containing β-Se-Fuc. (D) Sequence alignment among six fucose-binding sites was performed using the MAFFT program [34]. Similarity and secondary structure was determined using the ESPript 3 program with default settings [35]. Red characters with blue frames represent conserved residues. The green filled circles show residues located at equivalent positions in the fucose-binding sites that contribute to hydrogen bonds with the fucose moiety, with the exceptions of Y252 and S300. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)