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Fe-S cluster binding mechanism of pigeon's ISCA1 clarified by SEC-SAXS

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1 Introduction

The iron-sulfur cluster assembly 1 homolog clISCA1 of *Columba livia* (pigeon) interacts with a quantum biosensor protein clCRY4 [1]. The clCRY4/clISCA1 complex tends to orientate along the weak external magnetic-field lines (0.4–10G) under blue light, suggesting that clISCA1 might assist the function of clCRY4. Previously, we clarified that clISCA1 forms two types of protomers (the globular Type-A and the rod-like Type-B), and the Type-A protomer self-associates to form a columnar oligomer [2]. Moreover, it was expected that the Fe-S cluster binging to clISCA1 might improve the magnetic property of clISCA1. In order to elucidate the Fe-S cluster binging mechanism of clISCA1, we conducted the small angle X-ray scattering analysis coupled with size exclusion chromatography analysis (SEC-SAXS) and UV/Vis spectroscopy.

2 Experiment

SEC-SAXS data were collected with the ACQUITY UPLC H-Class (Waters, Massachusetts, USA), a Superose 6 Increase 10/300 gl column and a temperature-controlled flowthrough cell (20 °C) that allows UV-vis measurements directly on the SAXS exposure volume. The wavelength used was equal to 1.5 Å. The sample-to-detector distance was 2 m. In the SEC-SAXS measurement, 0.5 ml of 6.9 mg ml^{-1} clISCA1 in 20 mM Tris-HCl buffer at pH 8 including 0.15 M NaCl and 10 μM 3-Mercapto-1-propanol was loaded on the column. The flow rate was set to 0.4 ml min^{-1} and decreased to 0.05 ml min^{-1} when the protein eluted from the column to ensure long enough exposure times and accordingly better counting statistics in the obtained data. The SEC-SAXS images corresponding to 20 s of exposure were collected during the elution. Buffer scattering was subtracted from scattering curves of each fraction to yield the sample scattering curve using the program *SAngler* ver. 2.1.33. The averaged gyration radius *Rg* of eluted components in each fraction was evaluated from the scattering curves at the fraction frame No. 270–330 with the Guinier analysis using the program *Serial Analyzer* ver. 1.3.0.

Moreover, according to the procedure described in [2], the volume fractions of the clISCA1 components (the Type-A protomer model, the Type-B protomer model, and the oligomer models constructed from those protomers) in each fraction were evaluated by the program *Oligomer* [3].

3 Results and Discussion

The SEC-SAXS elution profile derived from the forward scattering $I(0)$ and UV absorbance at A_{280} of clISCA1 showed a broad peak at elution No. 240–340 (Fig. 1A). The *Rg*s evaluated from $I(q)$s of the elution No. 270–330 with the Guinier analysis decreased from 21.3 to 17.6 Å with the elution progress.

For the oligomer analysis, the theoretical scattering curves ($I_m(q)$s) calculated from the model structures showed good fitness for the experimental $I(q)$s at the elution No. 270–330 (Fig. 1B). Fig. 1C shows the volume fractions and shapes of the eluted components estimated by the oligomer analysis. The Type-A monomer was 44.2%–73.2% at the elution No. 270–330. The Type-A dimer was 4.1%–22.0% at the elution No. 270–284, 2.8% at the elution No. 296–300, and 2.0%–7.2% at the elution No. 322–330 (Fig. 1C). The Type-A tetramer or larger oligomers were 1.6%–15.4% at the elution No. 270–302. The Type-B monomer was 6.0% at the elution No. 270 and 2.8%–52.8% at the elution No. 286–330. The Type-B dimer was 5.0%–13.1% at the elution No. 270–274 and 1.5%–22.5% at the elution No. 278–324. The Type-A/Type-B complex and the Type-B oligomer larger than dimer were not detected in all elution ranges.

The UV/Vis absorption spectra of clISCA1 were also measured simultaneously with the SEC-SAXS measurement (Fig. 2). The absorption peaks at 330 and 420 nm reflecting the Fe–S cluster's existence [1] were obviously larger at the elution No. 270–290 than at the elution No. 295–330. Taken together with the result of the oligomer analysis (Fig. 1C), the UV/Vis absorption spectra indicate that the clISCA1 oligomers consisting of the Type-A protomer can bind Fe–S clusters and that the Type-A monomer, the Type-B monomer, and the Type-B dimer cannot bind Fe–S clusters. Therefore, the affinity of clICSA1 to the Fe–S cluster depends on the oligomeric state and the protomer structure of clISCA1.

A previous study indicated that the interaction between clCRY4 and clISCA1 is nearly abolished when the Fe–S cluster binding ability of clISCA1 is lost by the C60A/C124A/C126A mutation [1]. Since the release of Fe–S clusters occurs cooperatively with the conformational change of clISCA1 from the Type-A protomer to the Type-B protomer (Fig. 1C and 2), it can be considered that the clCRY4/clISCA1 interaction also needs the Fe–S cluster bound form of clISCA1, namely, the Type-A protomer.

The 2Fe-2S cluster or 4Fe-4S cluster binding site on protein molecules is formed by four side chains of Cys residues [4]. Since one clISCA1 molecule has only three Cys, the Fe–S cluster binding site formation on clISCA1 needs the assembly of more than two Type-A protomers. Therefore, the self-association of Type-A protomers to generate a columnar oligomer would make it possible to build Fe–S cluster binding sites between clISCA1 molecules. Periodic and regular binding of Fe-S clusters along the long axis of the columnar oligomer may improve the magnetic susceptibility and the magnetic anisotropy of the clISCA1 oligomer and the clCRY4/clISCA1 complex.

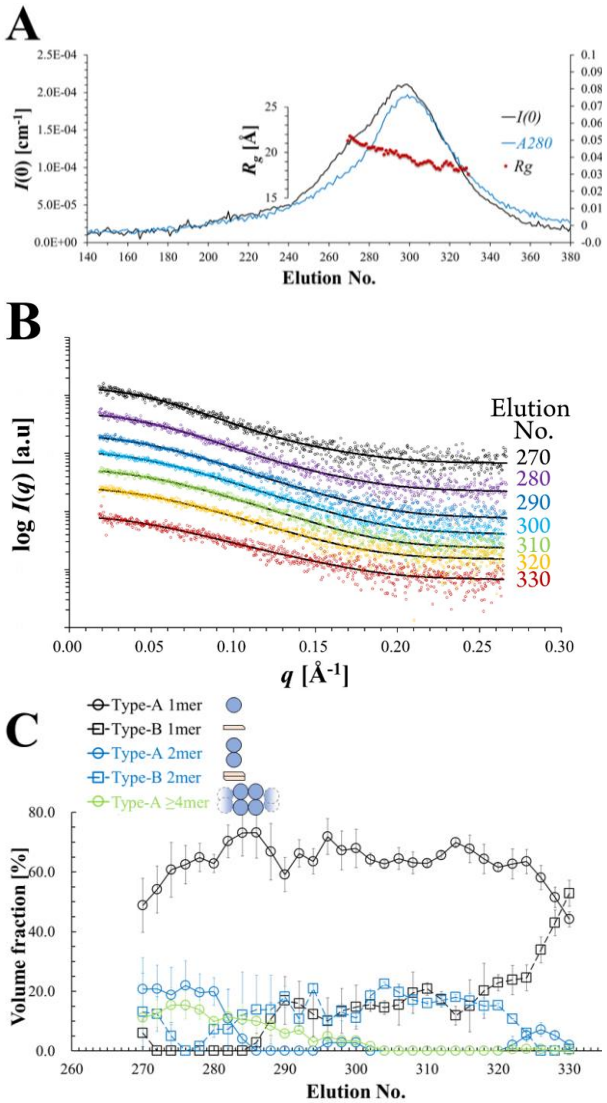

Fig. 1 SEC-SAXS data and oligomer analysis of clISCA1. (A) SEC-SAXS elution profile of clISCA1 (line). Dots represent Rg evaluated by the Guinier analysis. (B) The experimental $I(q)$s at the elution No. 270–330 (dots) and the theoretical $I_m(q)$s calculated by the oligomer analysis (lines). (C) The volume fractions of the components at the elution No. 270–330.

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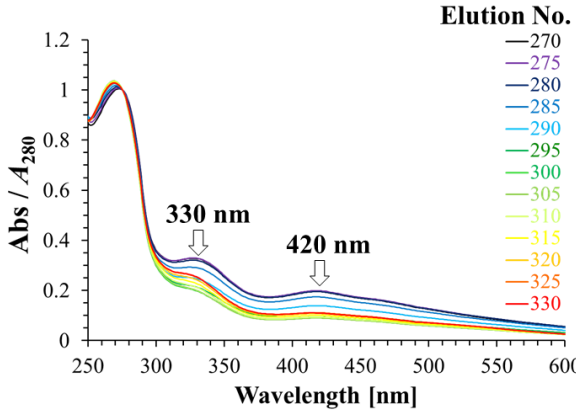

Fig. 2 The UV-vis absorption spectra of clISCA1 during the elution of SEC corresponding to the elution No. 270–330 of SEC-SAXS. These spectra were normalized by the absorption value at 280 nm.