BL-6A/2018G526

Attempt to observe the structure of schizophyllan monomer by Small Angle X-ray Scattering and Molecular Dynamics simulation

Yoshitaka Matsumura* and Masaki Kojima
School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

1 Introduction

Schizophyllan, a β-glucan derived from *Schizophyllum commune*, has been approved as an anticancer agent because it exhibits antitumor activity. The X-ray crystal structure analysis revealed that it takes a triple helix structure in the native state [1, 2] . On the other hand, it has been reported that the sonication of schizophyllan reduced the molecular weight and caused a structural change from trimer to monomer, resulting in loss of anticancer activity [3]. So, we aimed to observe and visualize the three-dimensional structure of the monomeric schizophyllan produced upon sonication by small angle X-ray scattering (SAXS) and molecular dynamics (MD) simulation.

2 Experiments

All the SAXS experiments were carried out at the beamline 6A using a PILATUS detector. The measurements were repeated under equilibrium at 25℃ with the sample-to-detector distance of ca. 0.5 m. For the Guinier analyses, both single and multiple exponential approximations were adopted [4, 5]. When irregular aggregates were contaminated in the sample solution, the multiple exponential approximation is very convenient and useful to obtain the structural properties of the major component in the mixture as was previously reported for other samples [5]. Bovine serum albumin and hen egg white lysozyme were employed as a reference for the calibration. Before the SAXS measurement, the solution of schizophyllan was sonicated for at least 200 hours. The sample concentration was less than 10 mg/mL. Molecular dynamics simulation was performed by Amber program package [6].

3 Results and Discussion

Figure 1 shows the Guinier plot (a) and the Kratky plot (b) of schizophyllan upon sonication. The Guinier plot indicated that the schizophyllan molecule was in a multi-dispersed state, and the obvious peak in the Kratky plot suggested a globular shape. The estimated radius of gyration (R_g) of the major component was almost the same (ca. 10 Å) in the Guinier and Kratky plots at several concentrations. Compared of its molecular weight from the Guinier plots with those reported previously, the major component in the current study could exist as a monomer [3]. The plausible structure was predicted by MD simulation using Amber with the molecular weight and the primary structure of the monomeric form obtained from the Guinier analyses. The resultant structure by MD simulation is shown in Figure 2 (a) and (b), which respectively show the stick model and the trace of the backbone. The backbone trace depicted a helical structure. Figure 2 (c) shows a coarse-grained structure of the major component of the schizophyllan constructed by *ab initio* modeling [7]. When the MD structure was aligned along the same inertial axis of the *ab initio* model, both structures were well superimposed (Figure 2 (d)). This indicates that the MD structure satisfied the SAXS structural information.

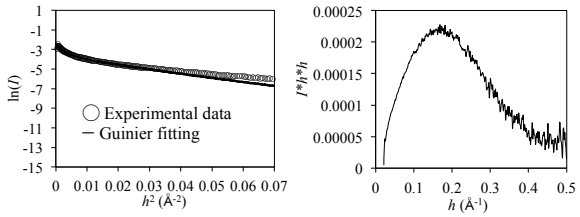

Figure 1. Guinier plot (a:left) and Kratky plot (b: right) of schizophyllan

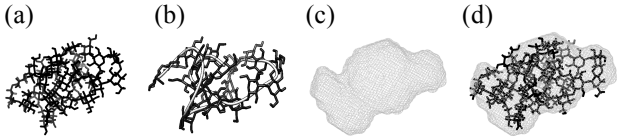

Figure 2. (a): Stick representation of the predicted structure from MD simulation, (b): The backbone trace of the structure (a), (c): coarse-grained structure from *ab initio* modeling, (d): superimposed structures (a) and (c).

References

[1] Y. Takahashi, *et al*. Rep. Prog. Polym. Phys. Jpn. 27, 767, (1984)
[2] D. B. Kony, *et al*. Biophys. J. 93, 442-455, (2007)
[3] T. Yanaki, *et al*. Biophys. Chem. 17, 337-342, (1983)
[4] A. Guinier, and G. Fournet, Wiley New York, (1955)
[5] Y. Matsumura, *et al*. Biophys. Chem. 175-176, 39-46, (2013)
[6] R. Salomon-Ferrer, *et al*. WIREs Comput. Mol. Sci. 3, 198-210, (2013)
[7] D. Franke, and D. I. Svergun, J. Appl. Cryst. 42, 342-346, (2009)

Outcome

Speaker, The 6th International Symposium on Bioimaging, The 28th Annual Meeting of Bioimaging Society, 2019

* matsumuy@toyaku.ac.jp