

Aggregates structural study of novel and potent immunosuppressant FTY720 in solution

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Introduction

FTY720, 2-amino-2-(4-octylphenyl)ethylpropane-1,3-diol hydrochloride, was synthesized with chemical modification technique as one of ISP-I, isolated from *Isaria sinclairii*, derivatives [1].

FTY720 prolonged rat skin allograft survival more effectively than cyclosporin A and showed activity in other administration routes [2]. FTY720 sequesters circulating mature lymphocytes into peripheral patches by acceleration of lymphocyte homing and thereby decreases the number of lymphocytes in peripheral blood [3]. FTY720 also had no inhibitory effect on serine palmitoyltransferase at a concentration of 1000 nM or less, suggesting that FTY720 possess considerable activity and is expected to be useful as an immunosuppressive drug for organ transplantation [2].

FTY720 consists of a hydrophilic group (2-amino-propane-1,3-diol) and a hydrophobic group (hydrocarbon chain and phenyl ring) in a molecule as is shown in Fig. 1. However, FTY720 in aqueous solution showed higher molecular weight by gel filtration method than monomer whose molecular weight was, (m/z 307(M^+) and $C_{19}H_{33}NO_2$), according to mass analysis of this sample.

In the present paper, we investigated the detailed molecular structure of aggregates of FTY720 in aqueous solution with Small-Angle X-ray Scattering (SAXS) using Synchrotron Radiation Source.

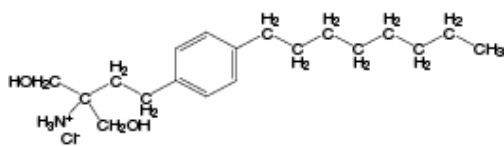


Fig. 1 Secondary structure of FTY720

Experimental

FTY720 was prepared as hydrochloride salt according to the method by Fujita et al. [2]. SAXS experiments were performed with the solution of FTY720 from 15 to 20 mg/ml in distilled water. The apparatus constant was obtained by using a solution of bovine serum albumin whose molecular weight was taken as 65,800.

SAXS measurements were carried out by using the optics and detector system SAXES (Small-Angle X-ray Scattering Equipment for Solution) were installed at BL-10C in the Photon Factory, KEK.

Results and Discussion

Fig. 2 shows the angular dependency of the scattering intensity profile of SAXS in FTY720 solution. Open circles indicate the experimental values. The theoretical curve as the two concentric spherical shell model is shown with the solid line taking account of the spherical factor, $P(Q)$.

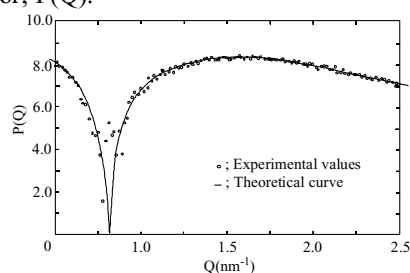


Fig. 2 Angular dependency of the scattering intensity profile of SAXS in FTY720 solution

Fig. 3 shows the Kratky plot of FTY720 solution of SAXS pattern. This plot showed one sharp peak of scattering curve, indicating that FTY720 aggregate is approximated as spherical shape.

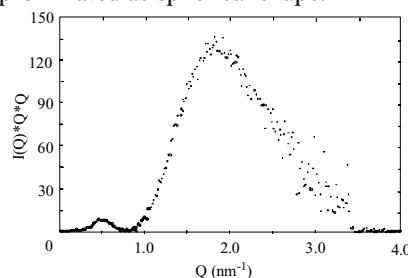


Fig. 3 Kratky plot of FTY720 solution of SAXS pattern

Angular dependency of the intensities of the scattered light in SAXS showed a typical pattern of the concentric spherical shell. The best fitted values of the theoretical curve as the two concentric spherical shell model was 0.8 nm for the inner radius and 3.6 nm for outer radius.

References

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- [3] K. Chiba et al., *J. Immunol.* **160**, 5493 (1998).

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