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Oligomeric structure of CEL-III, a hemolytic lectin from sea cucumber *Cucumaria echinata* in the presence of surfactants

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Introduction

CEL-III is a Ca^{2+}-dependent, Gal/GalNAc-specific lectin purified from sea cucumber *Cucumaria echinata*, which shows haemolytic activity, especially toward human and rabbit erythrocytes [1]. Hemolysis is caused by the colloid osmotic rupture of the erythrocyte membrane due to the formation of ion-permeable pores by CEL-III oligomer after it has bound to carbohydrate receptors on the cell surface. Oligomerization of these proteins can be induced not only in lipid membranes but also in solution under the appropriate conditions. CEL-III forms an oligomer in solution when complexed with lactose at high pH values and in the presence of high concentrations of salt, e.g. at pH 10 and with 1 M NaCl (artificial oligomer; a-oligo) [2]. Previous report shows that the molecular mass of the a-oligo is determined as 1019 kDa from its forward scattering value by small-angle X-ray scattering (SAXS) which is much larger than that estimated using SDS-PAGE, 270 kDa. [3]. Hence, SAXS measurements were carried out in the presence of surfactant to resolve the oligomeric structure.

Materials and Methods

CEL-III was purified from the body fluid of *C. echinata* using column chromatography on lactosyl-Cellulofine, GalNAc-Cellulofine, and Sephacryl S-200 as previously described [4]. The CEL-III oligomer was prepared by incubating it with 10 mM lactose at room temperature for 60 min in 20 mM glycine-NaOH buffer, 1 M NaCl, 10 mM $CaCl_2$ at pH 10. The remaining small amount of monomer was removed by gel filtration on a Sephacryl S-200 column. SAXS measurements and analysis were described elsewhere [5].

Results and Discussion

CEL-III monomer was purified from *C. echinata* successfully and oligomerized under the appropriate condition. SAXS analyses showed that the R_g,z values were 24.5, 99.0 and 62.1 Å for monomer, a-oligo and oligomer in the presence of 0.1% triton X-100 (t-oligo) (Fig. 1 (a)). Molecular weight calculated by the forward scattering intensity showed the a-oligo dissociate into hexamer from 25-mer of monomer in the presence of surfactant. This result well agreed with the result of SDS-PAGE. Kratky plot of a-oligo and t-oligo showed that the two peaks which is typical for oligomeric proteins (Fig. 1 (b)) [6]. α-hemolysine, one of the pore forming proteins associates into heptamer on the cell surface [7]. These results suggest that the oligomeric structure in the presence of surfactant is a minimum unit of hemolytic activity and a-oligo is consists of 4 t-oligos by weak interactions.

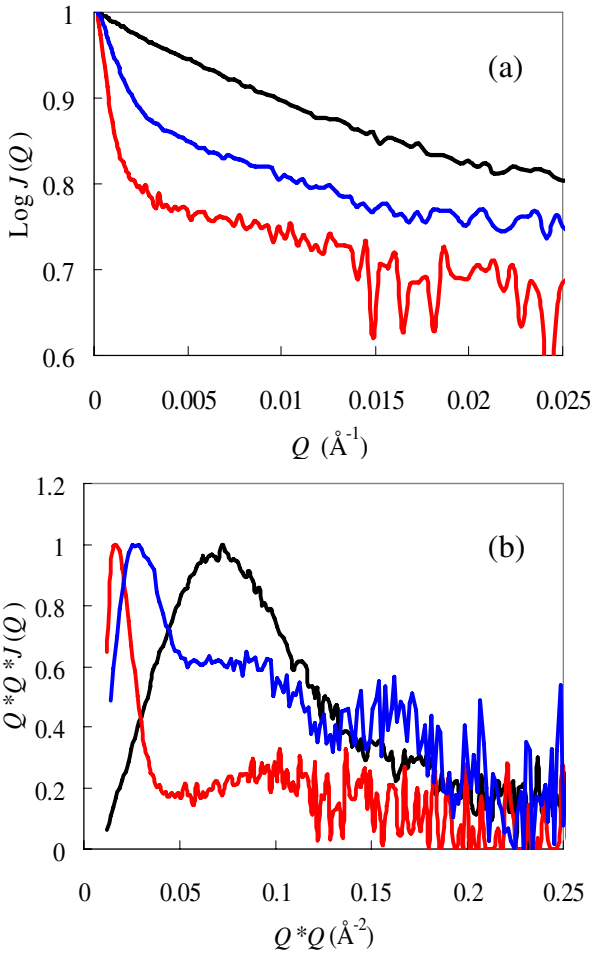

Figure 1. Guinier (a) and Kratky (b) plot of the CEL-III monomer (Black), oligomer (Red) and oligomer in the presence of 0.1% triton X-100 (Blue).

References

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