Supramolecular assembly of peptide molecules for applications in biological multivalent interactions

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Introduction

Multivalent interactions, which are characterized by the simultaneous binding of multiple ligands on multiple receptors, are prevalent in biological system. They have a number of characteristics that monovalent interactions do not. Multivalent interactions are nature's way of enhancing binding affinity between two entities in which the affinity increase can theoretically be as much as square of the respective binding constants.

Self-assembly is a bottom-up approach in which individual building blocks gather together to form an elaborate supramolecular aggregate. By spontaneously assembling each monovalent ligand, an object displaying multiple ligands can be realized.

In this report, we present an approach for building supramolecular objects where the driving force for the assembly is β-sheet interaction. We designed peptide-based monomers that are consisted of a functional block, which is for biological recognition, and an assembly block that has a propensity to form β-sheet assembly. One example is a monomer having α-D-mannose as the functional block and $(FKFE)_2$ peptide as the assembly block. Multiple hydrophobic and ionic interactions within the assembly block promoted the β-sheet packing of the monomeric units that resulted in the α-D-mannose-coated supramolecular objects. The specific and high affinity recognition of the multiple carbohydrate moieties within the objects with *E. coli* harboring binding partners for the specific carbohydrate is described.

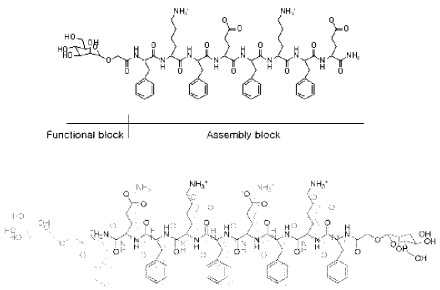

Figure 1. A building block [Mannose-$(FKFE)_2$] for the carbohydrate-coated supramolecular assembly and a proposed assembly model.

Experimental

Synthesis of the building block. The peptide building block were synthesized on Rink amide MBHA resin using standard Fmoc protocols. For coupling a functional mannose unit at the N-terminus of the peptide, carboxymethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside was coupled to the resin-bound peptide with HATU/DIPEA/NMP activation. The molecule was purified by reverse phase HPLC (water/acetonitrile with 0.1% TFA) and the purity was >95% as determined by analytical reverse phase HPLC.

Results and discussion

Cylindrical assembly of the peptide-based monomer. The formation of supramolecular assembly with propensity to form β-sheet structure was followed by circular dichroism (CD), dynamic light scattering (DLS), and transmission electron microscopy (TEM). CD spectrum of the monomer measured in aqueous solution showed a characteristic β-sheet structure with the negative ellipticity at around 215 nm. DLS measurement showed the presence of nano-aggregates with diameters of around 300 nm. These data suggest that mannose-$(FKFE)_2$ forms β-sheet-mediated intermolecular assembly leading to the building up of large nano-particles.

To characterized the morphologies of the aggregate, we investigated mannose-$(FKFE)_2$ assembly by TEM. Figure 2 shows TEM image of mannose-$(FKFE)_2$ after drying from an aqueous solution.

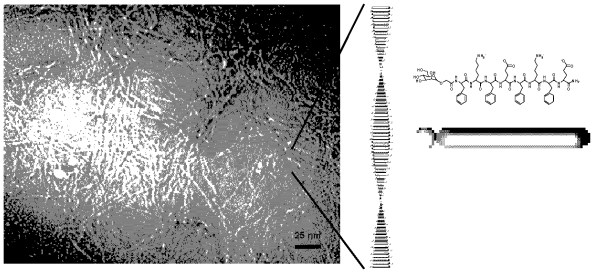

Figure 2. A TEM image of mannose-$(FKFE)_2$ and a proposed model of the assembly.

The TEM image showed the presence of long cylindrical aggregates with lengths up to several micrometers and widths of about 3 nm. The observed width is smaller than the molecular dimension of energy minimized structure (3.8 nm). The proposed model of the assembly is the twisted tape due to the intrinsic chirality of constituent peptide, leading to the decrease in the diameter of cylinders than the molecular dimension of the building block. The overall feature of the aggregate is a multiple mannose-coated long cylinder.

Specific multivalent recognition of bacterial cell. To show the usefulness of the assembly in biological multivalent interaction, we chose the interaction between the mannose units in the object and multiple mannose binding proteins (FimH) in the pili of *E. coli* ORN178 strain.

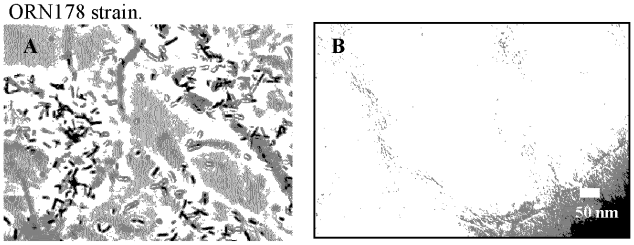

Figure 2. A phase contrast light microscope (A) and TEM images of agglutinated E. coli (ORN178).

Multiple FimH proteins are located along the long pili fiber and on the tip of the pili. The long cylindrical objects and the pili were interacted with each other by multivalent mannose and FimH interactions, thereby resulting in the aggregation or agglutination of *E. coli* cells (Fig 2A). TEM image showed that the objects (3 nm wide) and the pili (ca. 10 nm wide) were intertwined with each other, which explained the agglutination phenomenon. Monovalent mannose molecule didn't induce the agglutination showing the importance of being multivalent. An *E. coli* strain without mannose binding protein (ORN208) was not agglutinated by the object and high dose of monovalent mannose were able to induce disaggregation, showing the specificity of binding.

Conclusions

We have shown that it is able to make a supramolecular aggregate coated with multiple functional molecules fairly easily by simply mixing one building block. In this particular example, a mannose-coated object was able to agglutinate bacterial cells with cognate binding partners through multivalent interactions. This kind of strategy can be applied in developing materials that can selectively remove pathogens. The aggregate formed through β-sheet interaction should be stable and strong enough, and it was able to withstand the shearing force imposed by the large *E. coli* cells. Supramolecular assembly of this type should be very useful in exploring multivalent biological interactions.

References

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