Wrapping of Bio-macromolecules (Dextran, Amylopectin, and Horse Heart Cytochrome *c*) with Ultrathin Silicate Layer

Izumi Ichinose,[†,††] Yasuhiro Hashimoto,[†] and Toyoki Kunitake*[†]
[†]*Frontier Research System, RIKEN, 2-1 Hirosawa, Wako, 351-0198*
[††]*Advanced Materials Laboratory, NIMS, 1-1 Namiki, Tsukuba, 305-0044*

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We report a novel visualization technique of linear dextran and dendritic amylopectin structures by means of selective deposition of an ultrathin silicate layer along sugar chains. This technique was also useful for visualizing an isolated cytochrome c molecule.

Sugar chain plays significant roles in various biological events such as cell recognition, activation of growth factor, and in the infection of viruses on the surface of cells.[1] Understanding the interactions between sugar chains and proteins is one of the most important objectives in current molecular biology.[2] The sugar chain is, in general, composed of simple carbohydrate moieties which have hydroxy functional groups. Its structural diversity is tremendous because of chiral carbons, configurations of hydroxy groups, and versatility in polymerization of monosaccharides. These macromolecular structures have been studied mainly by accumulating biochemical evidences.[3] However, the molecular morphologies are still unclear, as compared to other bio-macromolecules such as nucleic acids and proteins.

Recently, we reported fabrication of ultrathin silicate layer around individual polymer chains.[4] This technique of "Molecular Wrapping" enabled us to physically isolate individual polymer chains from the external environment, as well as to observe each polymer chain by electron microscopy. In this paper we report extension of the wrapping technique to various biopolymers. Macromolecular structures of dextran and amylopectin were first directly observed by means of electron microscopy.

Aqueous sodium silicate (Na_2SiO_3) and dilute dextran solution were mixed with gentle stirring, and then a few tens μL of $Ba(NO_3)_2$ and $CsNO_3$ were added. The solution was allowed to stand at ambient temperature for 12 h. The concentration of dextran in the mixed solution was fixed to be 4.0×10^{-5} M in terms of α-D-glucopyranose unit, and the concentration of Na_2SiO_3 was 1.6 mM, forty times larger than that of dextran. A copper micro grid with 20-nm thick carbon film was placed on a droplet of the above mixed solution for 1 min, wiped from the side by using clean filter paper, and dried. Figure 1 shows a transmission electron microscope (TEM) image obtained from the mixed solution containing 0.8 mM $Ba(NO_3)_2$ and 0.8 mM $CsNO_3$. Observed were strand-like structures with a width of about 5 nm. The length appeared to be 50–300 nm.

We used dextran of average molecular weight of 66700. This molecular weight corresponds to glucopyranose units of about 410. The structure of dextran chain reported in literatures is composed of a linear main chain and short grafted chains of α-D-glucopyranose units (Figure 1).[5] When the dextran of 410 glucopyranose units is straightened out, the molecule length is expected to be 150 nm. This value is roughly consistent with the length of the strand-like structure in the TEM image. On the other hand, the width of the dextran chain should be in a range of 1.0–2.0 nm, since the size of α-D-glucopyranose is about 0.5 nm. The width of the strand-like structure (5 nm) is a few times larger than that expected from the dextran chain. However, if the chain is wrapped by a silicate layer with a thickness of 1.5–2.0 nm, the observed image is convincing. In our experiment, dextran was mixed with 40 times larger amount of Na_2SiO_3, and 20 times larger $Ba(NO_3)_2$ and $CsNO_3$ against glucopyranose unit. Deposition of these inorganic species causes increase in the apparent width of dextran chain.

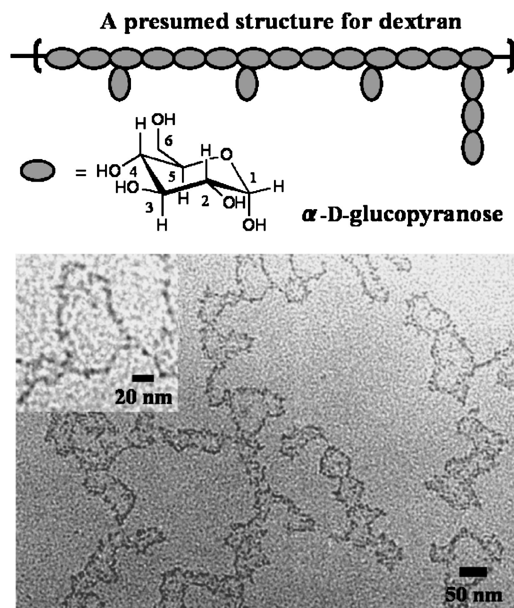

Figure 1. A presumed structure for dextran chain and TEM image of dextran wrapped with ultrathin silicate layer. The specimen was prepared from the mixed solution (pH 8.5) containing 4.0×10^{-5} M dextran, 1.6 mM Na_2SiO_3, 0.8 mM $Ba(NO_3)_2$, and 0.8 mM $CsNO_3$.

Sodium silicate (Na_2SiO_3) exists as negatively charged oligomers made of irregularly linked $–O–Si(O^-)_2–$ units. Shapes and sizes of the charged oligomers are diverse and changeable with the concentration and pH condition. The counter ions (Na^+) can be replaced by Ba^{2+} and Cs^+ ions, and these heavy ions act as effective imaging agents in TEM observation. Excessive Ba^{2+} ions are prone to agglomerate silicate oligomers. In fact, when the concentration of $Ba(NO_3)_2$ was 1.6 mM in the above mixture of Na_2SiO_3 and dextran ($CsNO_3$ was not added), granular aggregates of 20–40 nm in diameter were abundantly

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observed. On the other hand, when the concentration of $CsNO_3$ was 1.6 mM and no $Ba(NO_3)_2$ was added, we could observe neither granular aggregates nor strand-like structures. Proper adjustment of the concentrations of divalent Ba^{2+} ion and monovalent Cs^+ ion is indispensable for wrapping linear dextran chain with an ultrathin silicate layer.

Amylopectin and dextran are both composed of α-D-glucopyranose unit. However, in the first case, $\alpha 1 \rightarrow 4$ glucan chains are cross-linked through $\alpha 1 \rightarrow 6$ glucoside bonds, and, as a whole, highly branched structure is produced.[6] The degree of branching has been estimated from the restricted decomposition using hydrolase. We examined silicate wrapping to see the macromolecular structure of amylopectin. This sugar inherently tends to gelate. In order to increase the solubility of the wrapped amylopectin, we needed to add a small amount of sodium hydroxide into the mixture of Na_2SiO_3 and $CsNO_3$. Figure 2a shows a TEM image of amylopectin. The highly branched structure observed resembles the Gunja–Smith model that has been advocated long for branched amylopectin.[6] To the best of our knowledge, this observation is the first visual verification of branched amylopectin structure. It should be also emphasized that we could clearly distinguish between linear dextran and branched amylopectin chains.

Furthermore, our wrapping technique was applicable for horse heart cytochrome *c*, a water-soluble protein with molecular weight of 12384. Its dilute solution was mixed with an excess amount of Na_2SiO_3 with mild stirring, and the pH was adjusted to 8.5 with NaOH. After aging for 3 days, the solution was employed for TEM observation. As shown in Figure 2b, many granular structures with a diameter of 8 nm were observed by staining the specimen with 20 mM $Gd(NO_3)_3$. Some of them appear to be doughnut-like structure of 3.5-nm hole. The internal diameter was consistent with the dimensions of cytochrome *c* that has the size of $2.5 \times 2.5 \times 3.7$ nm.[7] Individual globular cytochrome *c* molecules must be wrapped with about 2.0-nm thick silicate layer. Cytochrome *c* is composed of 104 amino acids containing 18 lysine residues. The isoelectric point is at pH 10.6.[7] This protein is charged positively at neutral condition, and is able to electrostatically adsorb anionic silicate oligomers. pH value was very important for the isolation of each protein molecule. In fact, when the wrapping was conducted at pH 7.0, granular aggregates of 40 nm in diameter were abundantly observed. Successful wrapping was achieved at the pH a little bit lower than the isoelectric point of cytochrome *c*.

These studies provide the first demonstration of molecular wrapping for bio-macromolecules. It is striking that about 2.0-nm thick silicate layer deposits on the surface of sugar chain even in water. This means that the hydroxy surface can interact with silicate oligomers in spite of their strong hydration. Weak hydrophobic interaction or hydrogen bonding between sugar chain and silicate layer are probably enhanced by their macromolecular effect. Overall negative charges of wrapped sugar chains lead them to keep linear conformation. While the structural information of small bio-molecules can be studied by using X-ray diffraction or high-resolution NMR techniques, silicate wrapping is a convenient method for the visualization of much larger bio-molecular configuration. For example, images of large proteins, bio-molecular assemblies, and complexes of sugar chains and proteins would be obtainable. Site-specific binding of these macromolecules in aqueous media might be verified in the near future.

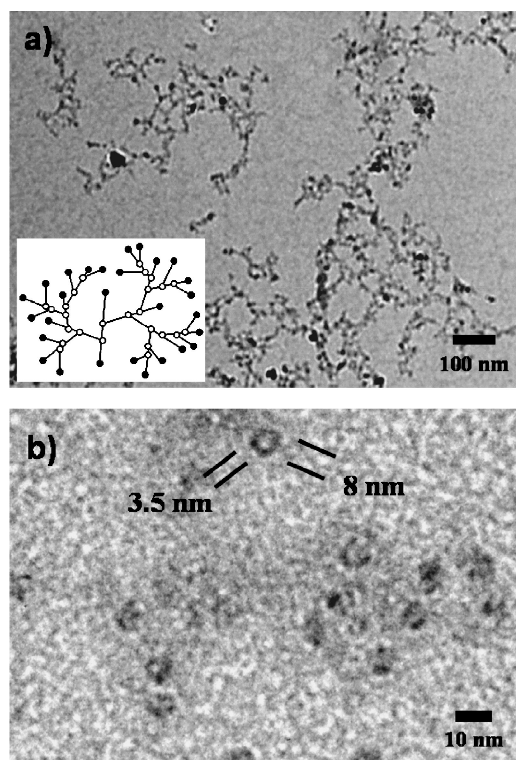

Figure 2. TEM images of amylopectin (**a**) and cytochrome c (**b**) wrapped with an ultrathin silicate layer. The insert is the simplified Gunja–Smith model for the structure of amylopectin. The former specimen was prepared from the mixed solution (pH 11.4) containing 1 mM amylopectin as α-D-glucopyranose unit, 20 mM Na_2SiO_3, 8 mM NaOH, and 40 mM $CsNO_3$. The latter specimen was prepared from the mixed solution of 1×10^{-5} M cytochrome *c*, 2 mM Na_2SiO_3, and 1 mM NaOH, and then stained with 20 mM $Gd(NO_3)_3$.

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