Aggregation of DNA-Modified Nanospheres Depending on Added Polynucleotides

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DNA oligomer, dT_{20}, was immobilized onto the organic nanospheres. The spheres aggregated only in the presence of complementary RNA under a special set of conditions. The aggregation was pseudo-reversible for the temperature change. The techniques of the polynucleotide-directed aggregation should be useful for constructing the structure having mesoscopic dimensions.

For the construction of mesoscopic structures, the specific base pairing of nucleic acids should be a powerful chemical adhesive of the building blocks. Recently, several groups succeeded in making unique structures using the complementary base pairing of DNA.[1-4] The well-defined rule of molecular recognition in double-stranded DNA (G-C and A-T base pairing) should promise "predetermined design" of the structure (Figure 1). In this context, constructing the structure through

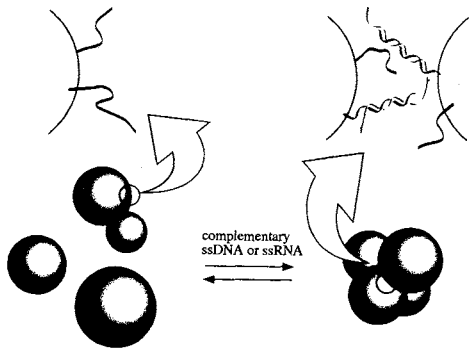

Figure 1. Controllable aggregation of organic nanospheres. The aggregation of the surface-modified nanospheres with oligonucleotides should be regulated by the several conditions such as added single-stranded DNA or RNA, temperature, and salts concentrations.

the nucleic acids base pairing would be in contrast with the aggregation in other self-assembled systems. This system also seems to be extendable toward a novel method for detecting mutant DNAs that does not rely on radio isotopes.[5-7]

In the present study, we have designed the pseudo-reversible system for attaining controllable aggregates using organic nanospheres on which certain oligonucleotide was anchored. Here, we report on the effect of added single-stranded nucleic acids (the averages length are *ca.* 300 bases) and some experimental conditions such as salt concentration and temperature on the aggregation behavior of the spheres.

5'-amino-terminated thymidylic acid 20 mer (**amino-T20**) was prepared on a fully automated DNA synthesizer. Carboxylate-modified polystyrene beads (40 nmϕ, Molecular Probes, Inc., FluoSphere) were used as nanosphere base in which red dyes (excitation /emission maxima = 580/605 nm) were pre-loaded. The **amino-T20** was anchored onto the spheres using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) in $NaHCO_3/Na_2CO_3$ buffer solution (pH 9.0).[8] After the capping of residual activated surface carboxylate groups with glycine, dT_{20}-modified nanospheres (**T20-sphere**) were isolated from free **amino-T20** and low-molecular weight by-products by GPC (Sephadex G-200). Surface coverage was roughly estimated from the amount of recovered unreacted **amino-T20**; one sphere had *ca.* 160 **amino-T20**s on its surface.

Light scattering in UV/Vis regions is a good measure for considering the aggregation size. The measurements were carried out using an ordinary spectrophotometer while mixing with an equipped stirrer. To begin with, salt concentration dependence of the **T20-sphere** aggregation was studied in the presence of 50 mmol dm^{-3} Hepes (pH 7.0) at 25 °C (data not shown). For the solution of **T20-sphere** alone and that with excess polyC or polyU (non-complementary RNA), an increase in the concentration of NaCl did not cause any change in the spectra. On the other hand, a transparent solution of **T20-sphere** with polyA (complementary RNA) rapidly became turbid in the NaCl concentration range higher than 1,000 mmol dm^{-3}. The same measurements at lower temperature required more concentration of the salt for aggregating.

These results indicated that the aggregation of **T20-spheres** proceeded only when a certain amount of NaCl, which decreases with rising the temperature, was added into the solution containing complementary single-stranded RNA. It is reasonable, because, in general, hybridization between polynucleotides is accelerated with an increase in salt concentration. In this case, we have to consider the properties of the sphere base as well; the aggregation would be suppressed by

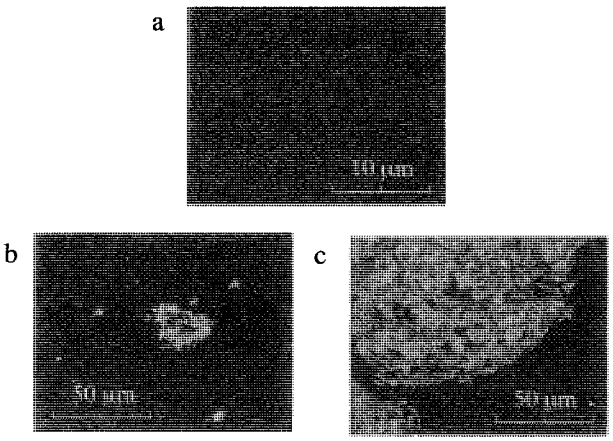

Figure 2. Fluorescence microscope images of **T20-spheres**. (a) dispersed **T20-spheres** in the presence of polyU. (b) the aggregate in turbid solution of **T20-sphere** in the presence of polyA (2.0 mmol-base dm^{-3}). (c) the precipitate from the turbid solution of **T20-sphere** in the presence of polyA. The gray spots on photos are the spheres luminous in bright red.

electrostatic repulsion between the spheres and the hydration of the sphere surface, both of which are also attenuated by increasing the amount of the salt.

Aggregation of the spheres was visualized by fluorescence microscopy equipped with appropriate filters (excitation/ emission = 510-550/>590 nm) and a CCD camera. All photos indicated in Figure 2 were taken at 25 °C in the presence of 1,300 mmol dm^{-3} NaCl and 50 mmol dm^{-3} Hepes (pH 7.0). Each of the spheres completely dispersed, and Brownian motion was observed in the presence of polyU (Figure 2a). On the other hand, the addition of complementary polyA made the spheres form many aggregates with dimension ranging from several to tens of micro meters (Figure 2b). The aggregates were easily precipitated by centrifuging (20,000 g, 30 sec) (Figure 2c). Less than 1.0 μdm^{-3} sample solutions were large enough for these observations.

Hybridization between polynucleotides is also sensitive to temperature. In the presence of 1,100 mmol dm^{-3} of NaCl, transmittance *vs.* temperature profiles were measured on the **T20-sphere** solution at 500 nm with a heating or cooling rate of 1.0 °C per minute. The changes in the transmittance in the presence of excess polyA and polyU are shown in Figure 3, with the spectra in the presence of polyA at 5 °C, 26 °C, and 70 °C obtained by separate experiments. Unexpectedly, it profiled unique unsymmetrically U-shaped curves. During the heating process, the transmittance was suddenly decreased at 22 °C and gradually increased from 40 °C. Finally the solution became fully transparent at 65 °C. The spectrum measured at each phase clearly indicates that the absorbance increase observed here is due to the light scattering, that is, the **T20-sphere** aggregated only in a certain temperature range; out of the range, no aggregation of spheres occurred. This behavior was pseudo-reversible for the temperature change. Further increasing the salt concentration widened the U-shape curve toward both sides. The **T20-sphere** solution containing polyC (data not shown) or polyU instead of polyA was wholly clear throughout the corresponding measurements. In addition, the aggregation in the presence of polyA was entirely suppressed by the addition of 5 mol dm^{-3} urea. Therefore, the observed aggregation is likely due to the specific bridging through the hydrogen bonding between complementary bases.

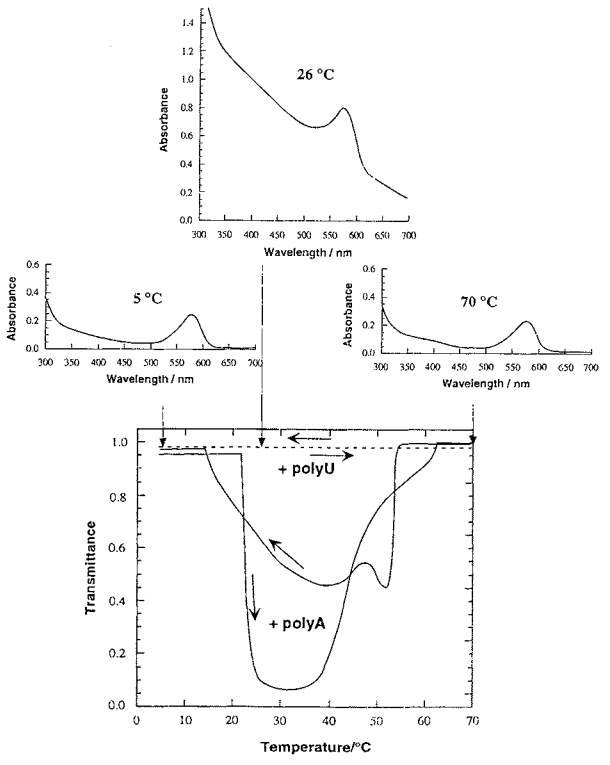

Figure 3. Aggregation behavior of the **T20-sphere** depending on the existing RNA homopolymers and the temperature. The temperature of an aqueous solution containing 1.0 x 10^{16} particles dm^{-3}, 1,100 mmol dm^{-3} NaCl, 50 mmol dm^{-3} Hepes (pH 7.0), and 2.0 mmol-base dm^{-3} RNA homopolymers was changed at the rate of 1.0 °C per minute. Solid and broken curves indicate the transmittance vs. temperature properties of the solution with polyA and polyU, respectively. The spectra were obtained by separate experiments at 5 °C, 26 °C, and 70 °C.

The equilibrium of polynucleotides' hybridization, duplex ⇌ coil lies well to the left at lower temperature.[9] It would be natural that the spheres aggregate at lower temperature and disperse at higher temperature. Presumably, hydration of the **T20-sphere**, which should be regarded as a hydrophilic colloid, may account for the observed stable dispersion of the spheres at a temperature below 20 °C. That is, although the duplex structure might be potentially dominant at the temperature, the hydration shell would prevent the spheres from direct contact with each other. According to the temperature rising, the hydrated water molecules are liberated from the surface of the **T20-sphere**. It then becomes barely possible to form the dT_{20}-polyA-dT_{20} cross-linked complex to give the aggregates at 22 °C. Subsequently, the aggregates re-disperse from 40 °C through the melting of the base pairing in the dT_{20}-polyA-dT_{20} complexes.

While characteristic interaction between DNA-modified Au nanoparticles was reported,[2] the system reported here is the first example of single-stranded RNA directed aggregation of DNA-modified organic nanospheres. Extension toward a multicolor system using the several colored spheres is in progress.

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