Polynucleotide Can Stabilize Nucleobase-appended Cholesterol Gels

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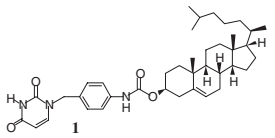
(Received December 13, 2002; CL-021059)

The influence of added poly(A) or poly(C) on the gelation ability of uracil-appended cholesterol gelator was investigated. It was found that RNA-gelator complexes were formed in *n*-butanol solution. In particular, complementary poly(A) not only stabilized the gel system but also created the helical structure in the original gel phase.

The recent focus of interest in supramolecular chemistry has been directed toward gelation properties of low molecular-weight compounds, because they not only gelate various solvents (including water) but also create a variety of supramolecular structures reflecting the molecular unit shape and the affinity with the used solvent. In many cases, the gelators result in the unique assembling structures such as tapes, rods, fibers, sheets, and cylinders which cannot be obtained from other systems.[1] Accordingly, it is now considered that gel chemistry is a new, attractive field of supramolecular chemistry.[1] To obtain such unique supramolecular structures and to stabilize the resultant gels, several research groups have designed and synthesized gelators having aggregative functional groups such as amides, ureas,[2] sugars,[3] steroids,[4] porphyrins,[5] etc. A function common to these groups is to facilitate the one-dimensional alignment of gelators by complementary intermolecular interactions. It is well-known that among these gelators, cholesterol-based gelators tend to form tightly packed columnar stacks, which are considered to be the origin of such one-dimensional fiber formation.[4] Functional groups appended to the C-3 position of the cholesterol skeleton are arranged, like a spiral staircase, around the central column. One may consider, therefore, that a cholesterol-based gelator is one of the ideal building blocks to organize the functional groups in a helical fashion. In fact, the cholesterol-based gel fibers frequently exhibit a helical structure due to its inherent chirality and packing mode. One may expect, therefore, that when some additive which can interact with the functional group in the gelator molecule is added, the morphology and the stability of the gel would be affected by this additive, leading to the creation of new supramolecular structures. In fact, it was already found that addition of certain metals,[6,7] donor/acceptor molecules,[8] polymers[9] etc.[5,10] does affect the morphology and the stability of the gels. It thus occurred to us that when polynucleotide is added to a nucleobase-appended cholesterol-based gelator systems, they might form a "double helix" consisting of the helical polymer (DNA or RNA) and the helical supramolecular aggregate (gel fiber).[11] To test this intriguing hypothesis, we initially designed and synthesized cholesterol-base gelator **1** which is functionalized by a uracil group as a recognition site.[12] One may expect for this uracil-appended gelator that when the cholesterol moieties aggregate into a one-dimensional columnar stack,[4] the uracil moieties are arranged like a spiral staircase around the column. As a result, the columnar aggregate would strongly interact with DNA or RNA and the thermal stability of the gel is sensitively affected by added polynucleotide. Here, we report our novel findings that the organogel sustained by **1** is remarkably affected by the addition of complementary poly(A) and that the resultant gel fiber shows a unique helical supramolecular structure.



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When the hydrogen-bonding guest is added to the original gel system, we sometimes encounter the difficulty to find an appropriate solvent which is miscible with both gelator and guest. In the present system, gelator **1** can gelate most organic solvents, whereas polynucleotide itself is virtually soluble only in water. Thus, we mixed *n*-butanol solution containing **1** with aqueous solution containing polynucleotide. The general procedure used for the gelation test is as follows. Gelator **1** (5.0 mg, 7.9×10^{-3} mol dm^{-3}) was dispersed in *n*-butanol (0.10 ml) and the mixture was warmed in a septum-capped test tube. This solution was immediately poured into the aqueous polynucleotide (Poly(A) or Poly(C)) solution (5.0 μl).[13] This treatment afforded a clear solution suitable for spectroscopic analyses. The water/*n*-butanol mixed solution (*n*-butanol:water = 20:1 (v/v)) was concentrated to dryness under reduced pressure for 1 h, and then *n*-butanol (0.10 ml) was added to the mixture (final concentration was adjusted to [**1**] = 5 wt%, 7.9×10^{-2} mol dm^{-3}). The solution was warmed until it became clear. After cooling to room temperature, the gel was subjected to the subsequent analyses.

The stabilities of organogels are usually evaluated with sol-gel phase transition temperature (T_{gel}).[1] The T_{gel} values were determined as a function of added polynucleotide concentrations. The results are listed in Table 1. It is seen from Table 1 that the gel is formed in the presence of polynucleotide, whereas **1** itself cannot form a stable gel under the same conditions. The T_{gel} values reach a maximum (76 °C) at [adenine base]/[**1**] = 0.3 molar ratio. At [**1**] = 7 wt% and 10 wt%, the T_{gel} values are further enhanced up to 82 °C and 91 °C, respectively. The findings indicate that **1** aggregates into a one-dimensional columnar stack with the aid of complementary hydrogen-bonding interactions between poly(A) and the uracil moieties in **1**.

In contrast to our expectation, however, we found that poly(C), the nucleobase of which is not complementary to **1**, is also effective to the gel formation. As shown in Table 1, the gel formation was recognized at [cytosine base]/[**1**] = 0.1 molar ratio and the T_{gel} values are almost comparallel to those of the **1**+poly(A) system. The finding implies that to stabilize the gel system, the formation of the hydrogen bonds between gelator and polynucleotide is crucial and they are not necessarily complementary.

We noticed, however, that the complementary versus non-complementary relationship appears in the gel fiber morphology. In

Table 1. Gelation test and T_{gel} values[a]

[**1**] wt%	[adenine base]/[**1**] molar ratio					
	0	**0.05**	**0.10**	**0.30**	**0.50**	**0.70**
3 wt%	**S**			**PG**		
5 wt%	**S**	**PG**	**G** (**70**)	**G** (**76**)	**G** (**72**)	**G** (**70**)
7 wt%	**G** (**80**)			**G** (**82**)		
10 wt%	**G** (**90**)			**G** (**91**)		
[**1**] wt%	[cytosine base]/[**1**] molar ratio					
	0	**0.05**	**0.10**	**0.30**	**0.50**	**0.70**
3 wt%	**S**			**PG**		
5 wt%	**S**	**PG**	**G** (**72**)	**G** (**69**)	**G** (**70**)	**G** (**70**)

[a]G = gel, PG = partial gel, S = solution, °C in the parentheses

the transmission electron microscope (TEM) observation, **1** in *n*-butanol solution, which gives the sol phase, results in spherical aggregates (data not shown here). When poly(A) was added ([adenine base]/[**1**] = 0.3 molar ratio), the aggregate structure has been changed to a well-developed fibrous network structure with 50–100 nm diameters and the solution was gelated (Figure 1A). The close-up picture reveals that most tape-like fibers are twisted in a right-handed helical fashion with 600–700 nm pitches (Figure 1B). Interestingly, we noticed that both the pitch length and the tape width becomes shorter with the increase in the poly(A)/[**1**] molar ratio: e.g., they are 20 nm diameter and 30 nm pitch at [adenine base]/[**1**] = 0.7 molar ratio (data not shown here). The similar phenomenon was previously observed by Huc et al. for cationic gemini surfactants having chiral tartrate counterions; the helical pitch becomes shorter with the increase in the e.e. of tartrate counter ions.[14] One may consider, therefore, that added poly(A) enforces the gelator molecules to be closely packed and to grow up as a helical structure.

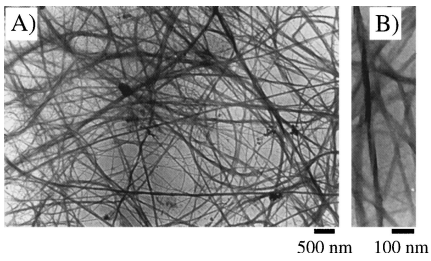

Figure 1. TEM images of the xerogel obtained from **1** (5 wt%)+poly(A)([adenine base]/[**1**] = 0.3) gel: stained with 2.0 wt% phosphotungstic acid.

In contrast, the **1**+poly(C) mixture gives a fibrous network but the helical structure is not found even in the close-up picture (Figure 2A,B). The finding implies that although the nonspecific **1**-poly(C) interaction is effective in the enhancement of T_{gel}, it is not so powerful as to induce the helical motif in the complexed supramolecular assembly. Only when the gelator nucleobase is complementary to that in poly-nucleotide, the well-ordered, periodical superstructure can appear.

A degree of nucleobase packing is conveniently monitored by a hypsochromic shift of the absorption maximum. In *n*-butanol solution, **1** (5 wt%) in the sol phase gives the λ_{max} at 244.0 nm whereas **1** in the gel phase (7 wt%) gives the λ_{max} at 243.2 nm. The slight but significant blue shift is attributed to the intermolecular stacking and hydrogen-bonding effects among uracil groups. When poly(A) or poly(C) was added to the gel (5 wt%, [adenine base] or [cytosine base]/[**1**] = 0.3 molar ratio), the λ_{max} further shifts to shorter wavelength, but the shift for the complementary poly(A) system (240.8 nm) is always larger than that for the noncomplementary poly(C) system (242.3 nm).

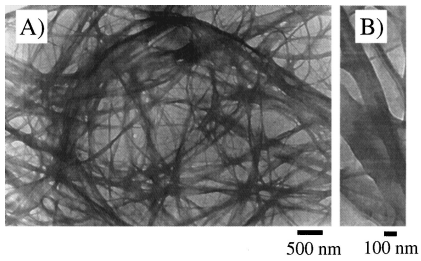

Figure 2. TEM images of the xerogel obtained from **1** (5 wt%)+poly(C) ([cytosine base]/[**1**] = 0.3) gel: stained with 2.0 wt% phosphotungstic acid.

In conclusion, we have found that when uracil-appended cholesterol gelator **1** is mixed with poly(A) or poly(C), added RNA strongly affects not only the T_{gel} values but also the morphology of formed gel fiber structures. In particular, the complementary poly(A) addition not only stabilizes the gel system but also induces the helical structure in the original gel fiber. Since polynucleotides are less soluble than **1** in *n*-butanol, one may consider that nucleotides occupy the inside and **1** molecules occupy the outside of the complex. Thus, the complex should have the tabacco-mosaic-virus-like superstructure. From these results, one may propose that the precise molecular recognition, like that in native DNA, could work even in the organogel systems.

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