

BCSJ Award Article

Delivery of Antisense Oligonucleotides to Nuclear Telomere RNA by Use of a Complex between Polysaccharide and Polynucleotide#

Jusaku Minari,[1] Takanori Kubo,[2] Hideki Ohba,[2] Naohiko Shimada,[1] Yoich Takeda,[1] Ryouji Karinaga,[1] Takahisa Anada,[1] Kazuya Koumoto,[1] Takeshi Kawazu,[3] Takeshi Nagasaki,[3] Seiji Shinkai,[4] and Kazuo Sakurai*[1]

[1]Department of Chemical Process and Environments, The University of Kitakyushu, 1-1 Hibikino, Wakamatsu-ku, Kitakyushu 808-0135

[2]On-site Sensing and Diagnosis Research Laboratory, AIST-Kyushu, Saga 841-0052

[3]Department of Applied and Bioapplied Chemistry, Graduate School of Engineering, Osaka City University, Osaka 558-8585

[4]Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka 819-0395

Received July 24, 2006; E-mail: sakurai@env.kitakyu-u.ac.jp

Telomerase, which is highly activated in neoplastic cells, can be a target for antisense therapy, and for that purpose, antisense oligonucleotides (AS ODNs) have to be effectively delivered into cellular nucleus where the target telomerase is present. The present work shows a new strategy to deliver AS ODNs to nucleus by use of a novel complex made from a natural polysaccharide schizophyllan (SPG) and AS ODNs. Nuclear transport is strictly regulated by the nuclear pore size and the related proteins. If the molecular weight of SPG is decreased, the SPG/AS ODN complex should be easily transported, although the stability of the complex decreases with a decrease in the molecular weight. We optimized the molecular weight of SPG to be 25 K. Furthermore, we attached importin-β (a nuclear transport protein) to the side chain of SPG by use of a streptavidin–biotin interaction. When this complex was added to Jurkat cells, the telomerase activity was more suppressed than the naked dose, indicating that the importin-β in the complex induced the nuclear transport of the complexed AS ODN and the AS ODN inhibited the telomerase. The present work shows a new methodology for nuclear anti-sense therapy that should be important in future anti-cancer therapies.

Telomere shortening determines cellular lifetime and eventually induces apoptosis; therefore, the activation of telomerase and the resultant extension of telomere are essential for immortalization of neoplastic cells. This fact implies that telomerase can be a target for anti-cancer therapy. Telomerase is a ribonucleoprotein complex containing an RNA subunit that serves as a template for reverse transcription of telomere DNA. Corey et al.[1,2] showed that the oligonucleotides (ODNs) complementary to the template RNA can block the transcription process and therefore suppress neoplastic growth. This event happens only when ODNs are effectively delivered into cellular nucleus, because the target telomerase is present in nucleus. This strategy can be regarded as an antisense therapy, although the target RNA is present in nucleus, while the target RNAs for conventional antisense therapy are present in cytosol. To make this strategy practicable, three issues should be considered: (1) improving instability of AS ODNs in biological fluids; (2) increasing the cellular uptake of AS ODNs; (3) delivering AS ODNs to target organelle.[3]

Instability of AS ODNs is ascribed to both deoxyribonuclease-mediated hydrolysis and non-specific binding to proteins.[4,5] The hydrolysis can be suppressed significantly by use of oligonucleotide analogues, such as phosphorothioate (PS) ODNs.[5,6] Interestingly, although the pathway is unknown, PS ODNs themselves have a peculiar ability to enter cells, which provides great advantage to this analogue.[7,8] On the other hand, the non-specific binding between PS ODNs and serum proteins has been pointed out as a serious problem.[9] It is reasonable to consider that this non-specific binding can be avoided (or suppressed) by using materials that can complex with PS ODNs.

Cationic lipids and synthetic polycations can form an ion complex with ODNs and encapsulate ODNs in the complex. There are many studies to show that these ionic complexes are applicable for antisense therapy to achieve the above-mentioned tasks.[5] Although the cations improve cellular uptake, they possess serious disadvantages, such as toxicity and poor solubility of the resultant polyion complexes, and these disad-

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vantages are mainly caused by the cationic nature itself.[10,11] These situations need an alternative material that does not use cation/anion interactions to form complexes with ODNs. We have found that a natural polysaccharide, called schizophyllan (SPG), can complex with single-stranded homo polynucleotides, without using cation/anion interactions.[12,13] Here, SPG consists of β-(1→3)-D-glucan main chain and one β-(1→6)-D-glycosyl side chain that links to the main chain at every three glucose residues, as shown in Fig. 3. The complexation occurs via a combination of the hydrogen bonding and hydrophobic interactions.[12–14] Subsequent studies have revealed that the SPG/ODN complexes reduce the hydrolytic degradation of the bound ODN and thus increase the antisense effect.[15,16] In addition, various functional groups to enhance the cellular uptake can be attached to SPG, including RGD and oligo arginine.[17,18] Mizu et al.[16] have shown that SPG can form a complex with PS ODNs in the similar manner with phosphodiester ODNs and the resultant complex can prevent the ODN from being absorbed by serum proteins. One interesting feature of the SPG/PS ODN complexes is that the ingestion ability, which PS ODNs inherently have, is scarcely affected.

The present work proposes a new method to deliver PS AS ODNs into nucleus to inhibit telomerase activity by blocking the template RNA. In order to provide a solution to the above-mentioned issues, we used a SPG/AS ODN complex, in which the SPG was chemically modified with importin-β beforehand. The complexation is expected to stabilize AS ODNs, and PS and importin-β are expected to increase the cellular uptake and nuclear transport, respectively.

List of Symbols and Abbreviations

SPG: schizophyllan. SPG/ODN complex: complex made from SPG and ODN with the molar rate of 1.5:1.0. PS ODN: phosphorothioate oligonucleotides. AS ODN: antisense oligonucleotides. RGD: Arg–Gly–Asp. Importin-β: a nuclear transport protein. tAS: antisense sequence for telomerase. The sequence is phosphorothioate 5′-CAGTTAGGGTTAG-(dA_{40})-3′. tRS: a scramble sequence of tAS, 5′-TAGAGCG-TATGTG-(dA_{40})-3′ phosphorothioate.

Experimental

Materials. Mitsui Sugar Co., Ltd., (Japan) kindly supplied a SPG sample with $M_w = 150$ K. The ODNs of 5′-CAGTTAGGGT-TAG-(dA_{40})-3′ and 5′-TAGAGCGTATGTG-(dA_{40})-3′ were used as an antisense sequence and its control, respectively. We attached a poly(dA) tail with 40 bases to the 3′ ends of these sequences to enhance the complexation, and these ODNs are denoted by tAS and tRS, respectively. Phosphorothioate tAS and tRS (liquid chromatography grade) were synthesized by Hokkaido System Science in Japan. Phosphodiester poly(dA_{40}) (unmodified, or rhodamine labeled) was used for circular dichroism (CD), dynamic light scattering (DLS), and microinjection. FITC (fluorescein isothiocyanate)-labeled phosphorothioate poly(dA_{40}) was used for confocal laser scanning microscopy. *N*-Succinimidyl 3-maleimidopropionate (SMP) and (+)-biotin *N*-hydroxysuccinimide ester (BNHS) were obtained from Tokyo Chemical Industry Co., Ltd. and SIGMA, respectively.

Preparation of Cells. NIH3T3 cells (mouse fibroblast) were grown in Dulbecco's modified Eagle's medium containing 10% FBS, 100 μg mL^{-1} streptomycin, and 100 U mL^{-1} penicillin at 37 °C in a 5% CO_2 atmosphere. The cultured cells were plated on a marked cover-slip for about 24 h before microinjection. Jurkat cells (human leukemia) were cultured in a RPMI-1640 medium supplemented with 10% FBS, 100 μg mL^{-1} streptomycin, and 100 U mL^{-1} penicillin at 37 °C in a humidified atmosphere containing 5% CO_2. The cells used for the biological assays were in a logarithmic phase. They were sedimented by centrifugation at 1000 rpm for 10 min and washed three times with PBS at 4 °C before the experiments.

Hydrolysis of SPG & Chemical Modification to the Side Chain. The acid hydrolysis of the main chain of SPG was performed according to the established method.[19] The selective oxidation of the 1,2-diol group and induction of 2-aminoethanol into the formyl terminate (see Fig. 3) were carried out with the methods described in the previous paper.[20] RGD peptide attachment was also descried elsewhere.[17,21,22]

Biotination and Importin-β Conjugation of SPG. 25 K-SPG-Am (42 mg) was dissolved in DMSO (8.4 mL), and 1.6 mL of DMSO/(+)-biotin *N*-hydroxysuccinimide ester solution (2 mg mL^{-1}) was added to the 25 K-SPG-Am solution. After 3 days with stirring under N_2, the product was dialyzed with a molecularporous membrane tubing (Spectra/Por, a 3500 cut-off) for 5 days. The lyophilization gave 42 mg of biotin-attached 25 K-SPG. From 150 K-SPG-Am, we obtained biotin-attached 150 K-SPG in the similar manner except for using a 14000 cut-off semipermeable membrane (Viskase Companies). The modification levels of biotin were determined with elemental analysis to be 14 and 12 mol % for 25 K-SPG and 150 K-SPG, respectively (Table 2). We mixed streptavidin-fused importin-β and biotin-attached SPG in an appropriate molar ratio so that one importin-β molecule should attach one SPG chain.

Complexation. The molar ratio of SPG repeating unit to ODN base number was always fixed at 1.5:1.0, which is an excess of SPG over the stoichiometric number.[13] The detail method of the complexation of ODN with SPG was described elsewhere.[12]

Telomerase Assays. Telomerase activity for Jurkat cells was detected using a conventional telomeric repeat amplification protocol (TRAP) and the stretch PCR method using a telomerase assay kit (TeloChaser; Toyobo Co., Ltd.). The products of the telomerase reaction (TRAP products) were analyzed by TBA electrophoresis in a non-denaturing 12% polyacrylamide gel. The gels were stained using a DNA silver staining kit (Pharmacia Biotech) according to the manufacturer's instructions. The control and negative control were no inhibition and addition of the TeloChaser lysis buffer (TOYOBO), respectively. The results were calculated as percentages of the positive control and normalized against the internal standard.

Other Measurements. CD,[12] microinjection,[23] and confocal laser-scanning microscopy[24,25] were performed, and those experimental details were described elsewhere. The hydrodynamic radius (R_h) in each SPG and the complex was determined using a Zetasizer Nano-ZS (Malvern Instruments Ltd.) with 100 mM Tris or 100 mM NaCl buffer at 37 °C. Microinjection assay was carried out in order to clarify whether the small complex can be transported through nuclear pore.

Result and Discussion

Molecular Weight Control of SPG Samples and Their Complexation Ability. An SPG sample, obtained from Mitsui Sugar Co., Ltd. (Japan), had a weight average molecular weight (M_w) of 150 K. Hydrolysis of this sample with formic acid produced 3 samples with a smaller M_w value as pre-

Table 1. Sample Codes and Their Molecular Characters

Sample code	Hydrolysis time /days	M_w	M_w/M_n	T_m /°C	R_h/nm before[a)]	R_h/nm after[b)]
150 K-SPG	as received	150000	1.47	43	37	26
25 K-SPG	4.5	25000	1.17	42	23	11
15 K-SPG	9.0	15000	1.44	broad[c)]	8	8
5 K-SPG	15	5000	1.22	—	NG[d)]	—

a) Before the complexation. b) After the complexation with poly(dA)$_{40}$. c) The transition of the CD_{250} curve was too broad to determine T_m. d) The size was too small to detect.

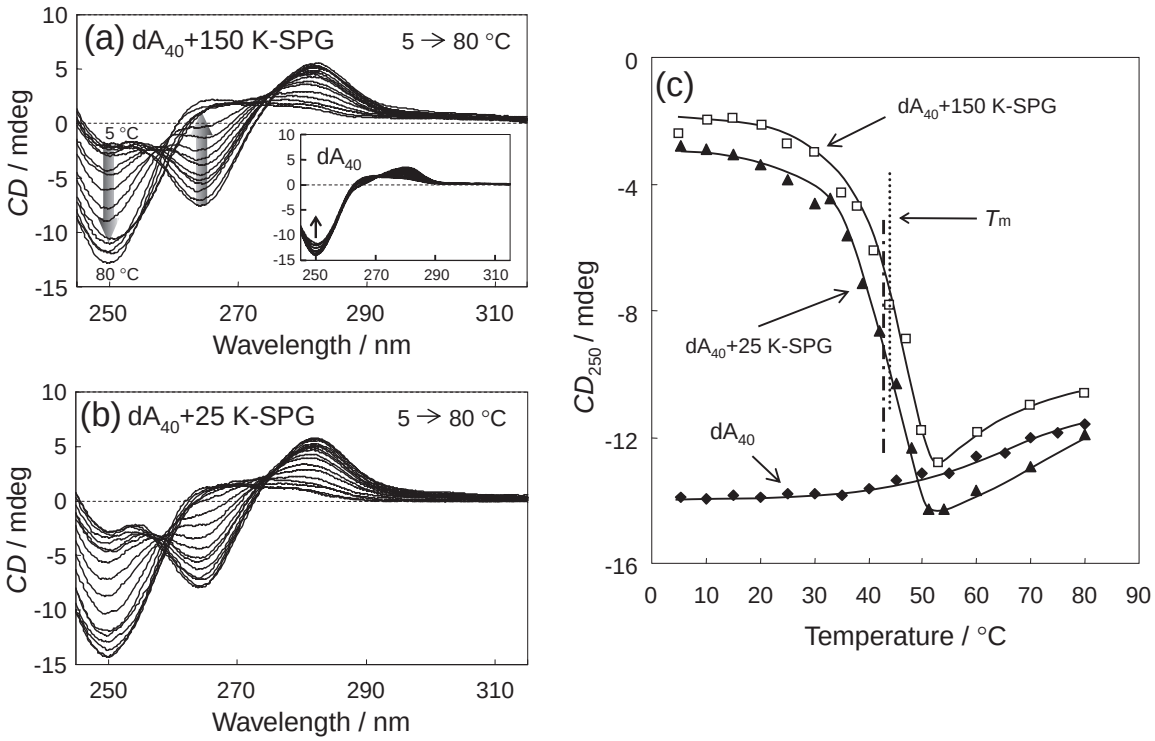

Fig. 1. Temperature dependence of CD spectrum for poly(dA)$_{40}$ itself (inset of the panel a), the mixture of poly(dA)$_{40}$ and 150 K-SPG (panel a), and the mixture of poly(dA)$_{40}$ and 25 K-SPG (panel b). Panel c is a plot of the CD values at 250 nm (CD_{250}) against temperature and the dotted lines show the dissociation temperatures (T_m). The arrows in (a) and (b) show the direction of the spectral changes upon heating.

sented in Table 1. With an increase in the hydrolysis time, M_w decreased and finally reached 5 K after 15 days of hydrolysis. An M_w value of 5 K corresponds to 24 glucose-residues in the main chain. Previous work confirmed that the hydrolysis did not affect the β-(1→6)-D-glycosyl side chain.[19]

The SPG chain has a minimum length in order to form the complex and above this critical length, the dissociation temperature of the complex increases with an increase in the chain length (i.e., the stability of the complex increases).[26] We mixed poly(dA)$_{40}$ with each SPG sample in Table 1 and examined whether they form complexes using CD. Figure 1a compares the temperature dependence of the CD spectrum between poly(dA)$_{40}$ itself (inset) and the mixture with 150 K-SPG. At low temperatures, the mixture showed completely different spectra from those of poly(dA)$_{40}$. This is because the complexation with 150 K-SPG altered the poly(dA)$_{40}$ conformation. As the temperature increased, the spectrum gradually came to resemble that of poly(dA)$_{40}$ itself. 25 K-SPG (Fig. 1b) showed the similar results with those of 150 K-SPG. Figure 1c shows a plot of the CD values at 250 nm (CD_{250}) against temperature comparing the mixture and poly(dA)$_{40}$. CD_{250} of the mixture merges into that of poly(dA)$_{40}$ around 50 °C, indicating that the dissociation of the complex took place in this temperature range. We defined the temperature that gave the maximum of the differentiated CD_{250} as the dissociation temperature (T_m), and the values of T_m are listed in Table 1. When M_w was less than 5 K, the complexation did not occur. Although 15 K-SPG formed a complex to some extent, the dissociation curve was too broad to estimate a clear T_m, indicating that the stability is too low to use this complex for biological assays.

Figure 2 compares the size distribution curves among 25 K-SPG, 150 K-SPG, and their complexes with poly(dA)$_{40}$, determined by using DLS at 37 °C, where the sample concentrations were about 10 times as high as those for transfection assay to obtain reliable data. On the basis of the stoichiometric number for the complex, all of the fed SPG (25 K-SPG and 150 K-SPG) was used in the complexation (see the experimental section). The complexation made the size smaller than that of

SPG itself.[27] The 6th and 7th columns in Table 1 compare the averaged hydrodynamic radius (R_h) obtained from Fig. 2. The value of R_h for the 25 K-SPG complex was 11 nm, which is less than the nuclear pore size (ca. 20 nm),[28–30] and therefore, this complex is expected to pass through the pore. On the other hand, the 150 K-SPG complex had a R_h value of 26 nm, which is comparable to or larger than the pore radius. It should be noted that, since DLS needs rather high concentrations and in this concentration range, importin-β forms aggregates, we could not accurately measure the size of the SPG/importin-β conjugate.

Chemical Modification of SPG. Since SPG is a neutral polysaccharide, it has no ability to enter cells unless a particular receptor that can recognize β-(1→3) glucans, such as Dectin-1, is present on the cellular surface.[31] In order for both ingestion and nuclear transport, we attached RGD and importin-β to the side chain of SPG. As mentioned in the introduction, PS ODNs can be ingested, and thus, by attaching RGD, we tried to increase further its ability to be ingested.

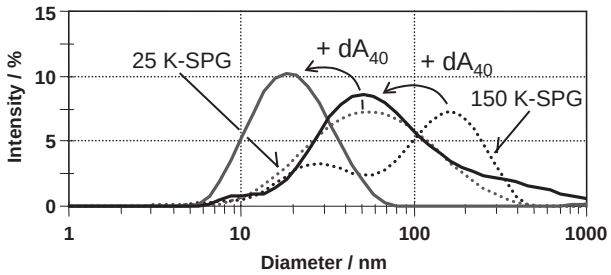

Fig. 2. Comparison of the size distribution curves among 25 K-SPG, 150 K-SPG, and their complexes with poly-$(dA)_{40}$, determined with DLS.

Koumoto et al.[20] and Matsumoto et al.[17] have reported that various functional groups can be attached to the side chain of SPG. According to them, it is important to introduce the functional groups only to the side chain in order to maintain the polynucleotide/SPG complex, because the β-(1→3)-D-glucan main chain is necessary to form the complex.[32,33] We used their procedure to attach RGD and importin-β to SPG as presented in Fig. 3. The key step was oxidation of the side-chain glucose by periodate anion (IO_4^-), which caused the cleavage of the pyranose ring and converted each end to the formyl terminate (step i in the figure). The formylation rate was controlled by the amount of sodium periodate ($NaIO_4$). After the formylation, an excess of 2-aminoethanol was reacted with the formyl group. The aminoethanol group was then used to attach RGD (iii and iv) or biotin (v). In this procedure, we carefully controlled the amount of each bifunctional cross-linker (SMP(iii) or BNHS(v)) to be less than that of the 2-aminoethanol terminate.[34] The aminoethanol, RGD, and biotin modification rates were determined with elemental analysis, and the results are listed in Table 2. We repeated the above procedure until 150 K-SPG and 25 K-SPG had nearly the same modifications.

RGD is a peptide that can bind to integrins, and integrins are a natural target for receptor-mediated endocytosis. Since receptor-mediated endocytosis ingests a substance better than regulatory pinocytosis, RGD-attached SPG was expected to enter cells more effectively. Importin-β plays an important role in nuclear transport.[35,36] Nagasaki et al.[23] were the first to prepare streptavidin-fused importin-β and conjugate it with biotin-attached plasmid-DNA. They have reported that the resultant importin-β increases the nuclear transport of the plasmid-DNA. In this paper, we applied their method to the SPG/ODN complex.

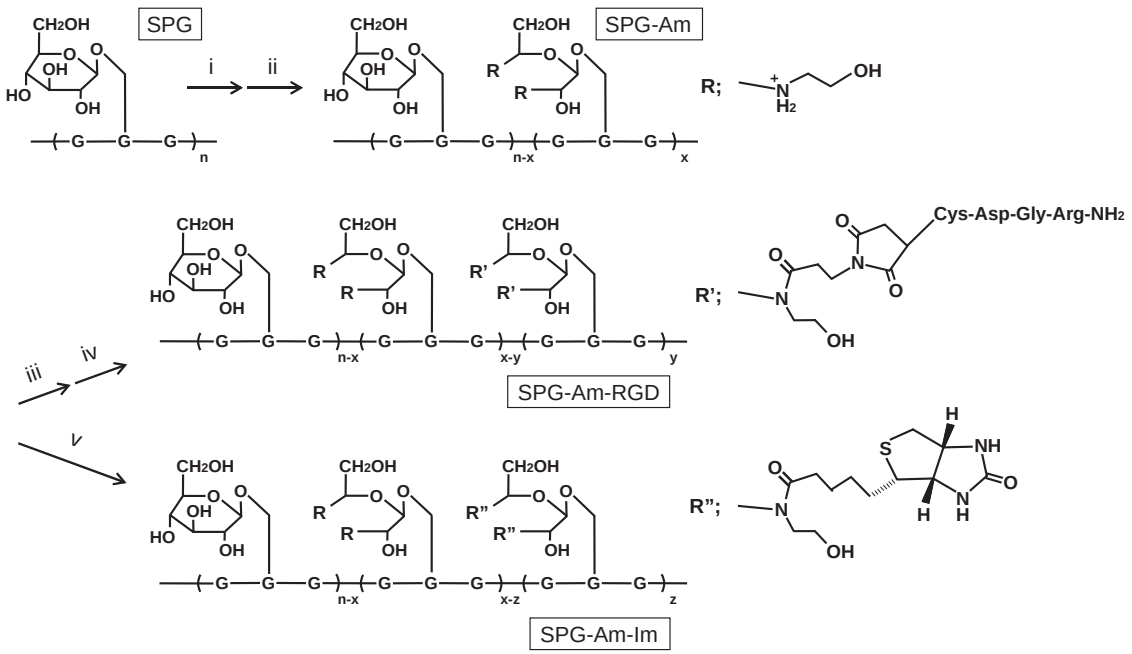

Fig. 3. Scheme illustration of the chemical modification of SPG. The reagents and conditions are as follows: (i) $NaIO_4$, H_2O, 4 °C, 3 days; (ii) 2-aminoethanol, DMSO, rt, 2 days, then $NaBH_4$, DMSO, rt, 1 day; (iii) *N*-succinimidyl 3-maleimidopropionate (SMP), DMSO, rt, 3 days; (iv) RGDC peptide, H_2O, rt, 3 days; (v) (+)-biotin *N*-hydroxysuccinimide ester (BNHS), DMSO, rt, 3 days. In the schemes, G stands for the glucose residue in the SPG.

Nuclear Transport of the SPG Complex Micro-Injected in Cytosol. Figure 4 compares the intracellular distribution of rhodamine-labeled poly(dA)$_{40}$, after each poly(dA)$_{40}$/SPG complex was micro-injected into the cytosol of NIH3T3 cells. For 25 K-SPG with no aminoethanol attached, the injected complex was distributed uniformly inside of the cell (b). For 25 K-SPG-Am (aminoethanol was attached), the complex seemed to be located more in the nucleus than in the cytosol (c). On the other hand, when 150 K-SPG without aminoethanol was injected (d), the complex was not localized in the nucleus, indicating that the complex is too large to enter the nucleus. Even after the introduction of aminoethanol, the complex was still present more in the cytosol than in the nucleus (e).

When the complex was made from cationized SPG with lower M_w, nuclear transport was enhanced (compare the photos c and e). This is consistent with the size selectivity and the cationic nature being favorable for nuclear transport. Nagasaki et al.[23] have reported a microinjection study for plasmid DNA/importin-β conjugate, showing that the conjugate is more localized in nucleus and thus has higher expression efficiency than intact plasmid DNA. We can expect the same effect for the importin-β conjugated SPG.

Table 2. Sample Codes for Modified SPG and Their Modification Levels

Sample Code	Aminoethanol /mol %	RGD /mol %	Biotin /mol %
25 K-SPG-Am	29	—	—
25 K-SPG-Am-RGD	24	5	—
25 K-SPG-Am-Im	15	—	14
150 K-SPG-Am	28	—	—
150 K-SPG-Am-RGD	24	4	—
150 K-SPG-Am-Im	16	—	12

Antisense Assay to Target Telomerase RNA in Nucleus. An antisense sequence (tAS) or its randomized sequence (tRS) were complexed with 25 K-SPG, 25 K-SPG-Am-RGD (RGD was attached), or 25 K-SPG-Am-Im (importin-β was attached) and exposed to Jurkat cells, and the complexes were compared to naked doses of tAS or tRS. Here, tAS was designed to hybridize with the template sequence of hybridized Jurkat's telomerase RNA.[24] After injection, the telomerase activity was determined with TRAP.[37,38] According to this protocol, the cell lysate was added to a varied number of telomeric repeats (TTAGGG), and PCR was used to amplify the extension products. The telomerase activity can be related to the amount of the extension products, which are observed on a native polyacrylamide gel.

The assay results are presented in Fig. 5. The controls (no inhibition, lanes 1 and 6) showed distinct ladders of the telomeric repeats; on the other hand, the negative controls (adding only the lysis buffer, see the experimental section) did not show any ladders. When tAS was added, the telomerase activity was slightly reduced, whereas no inhibition occurred when tRS was added. The numbers above each lane indicates the amount of telomeric repeats determined with densitometry. The comparison of these numbers shows that 25 K-SPG-Am-Im inhibited the telomerase activity more than the others. This result can be rationalized by the fact that the tAS bound in the SPG complex is protected from unfavorable interactions with proteins in the culture medium[16] and that nuclear transport is enhanced due to importin-β.

When the target was present in cytosol, attachment of RGD to SPG enhanced the antisense effect dramatically, as present-

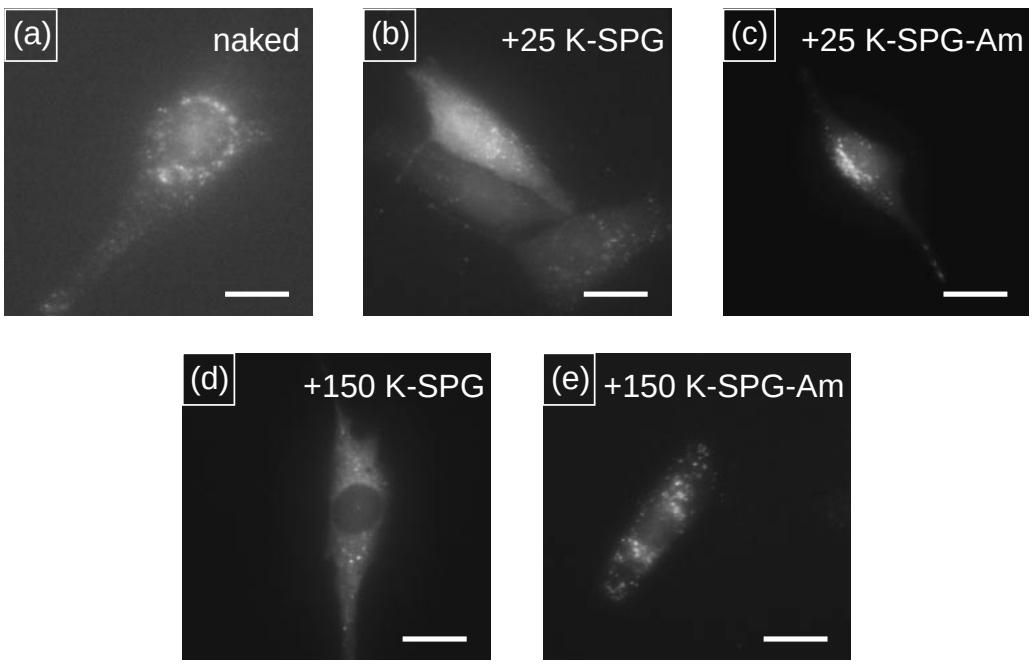

Fig. 4. The intracellular distribution of rhodamine-labeled poly(dA)$_{40}$ when poly(dA)$_{40}$ (1.5 μg μL^{-1}) was micro-injected into NIH3T3 cells after complexed with 25 K-SPG (b), 25 K-SPG-Am (c), 150 K-SPG (d), or 150 K-SPG-Am (e). The cells were incubated for 1 h and fixed with 3.7% formaldehyde (PBS). The nucleus was less stained in (d) and (e) compared with (b) and (c). In (c), the rhodamine dots were more localized in the nucleus than the other images. All scale bars are equivalent to 15 μm.

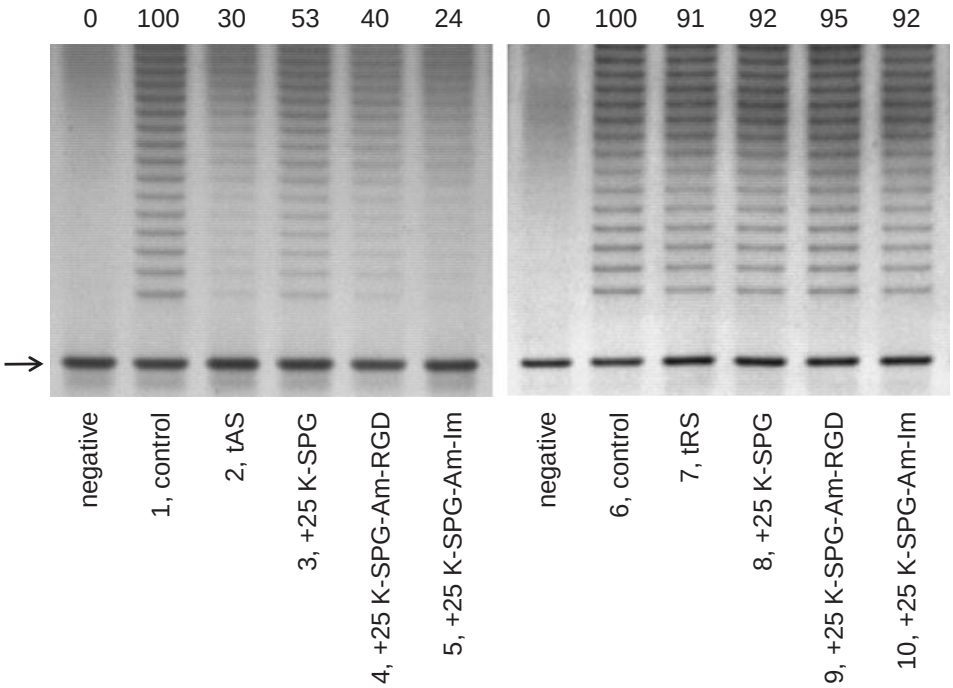

Fig. 5. Telomerase activity assay to show the inhibition of telomerase when tAS (5 μM) was administrated to Jurkat cells (0.5×10^6 cells mL^{-1}) at the naked state (lane 2), complexed with 25 K-SPG (lane 3), with 25 K-SPG-Am-RGD (lane 4), or with 25 K-SPG-Am-Im (lane 5). The lanes from lane 6 to 10 are their controls with tRS (5 μM). The cells were lysed at 48 h after transfection, and the telomerase activity was determined according to TRAP.

ed in our previous paper.[21] Furthermore, the simple complexation with unmodified SPG and PS ODNs increased the antisense effect for the melanoma cell lines of C32 and A375.[16] As shown in Fig. 5, RGD attachment and unmodified SPG did not increase the antisense effect in Jurkat cells.

As a matter of fact, our original aim was to inhibit the telomerase activity by use of a hybridized complex having both RGD and importin-β, which is similar to a PEG/RGD hybridized complex.[39] The antisense effect for RGD/importin-β hybridized SPG was always negative, showing poor inhibition and sometimes the same level as the control. We think that the positive RGD and negative importin-β form an ionic complex and thus both functions should be deactivated. Furthermore, we carried out the same assay using phosphodiester ODNs instead of PS ODNs and found that there was no appreciable inhibition, suggesting the importance of PS ODNs for cellular ingestion for Jurkat cells.

Further Evidence for Nuclear Transport. Transfection of ODNs into Jurkat cells is generally inefficient. In this sense, the ingestion ability of PS ODN presented in Fig. 5 is remarkable. The ingestion was not improved in the RGD system. To increase the cellular ingestion of the ODNs, a lipoplex was prepared from Lipofectamine™ 2000 and the SPG/ODN complex, and then, the resulting lipoplex which has no cytotoxicity in these conditions (see the Supplementary Information S3) was applied to Jurkat cells. In this case, the TRAP assay showed a dramatically increased inhibition (see the Supplementary Information S4). These results mean that Lipofectamine™ 2000 can induce cellular ingestion. Therefore, we decided to observe the intracellular distribution after transfection with Lipofectamine™ 2000.

Figure 6 compares the intracellular distribution for (b) naked FITC-poly$(dA)_{40}$, (c) 25 K-SPG/FITC-poly$(dA)_{40}$, (d) 25 K-SPG-Am-RGD/FITC-poly$(dA)_{40}$, and (e) 25 K-SPG-Am-Im/FITC-poly$(dA)_{40}$. Use of Lipofectamine™ 2000 caused a large amount of ingestion as presented; On the other hand, without Lipofectamine™ 2000, no ingestion occurred (see the Supplementary Information S5). In the un-complexed dose with SPG, it seemed that some amount of ODN was adsorbed on the cellular surface. These can be compacted complexes made from Lipofectamine™ 2000 and FITC-poly$(dA)_{40}$, and they might be absorbed on the cellular surface. Photo (c) shows that a small amount of unmodified SPG complex was ingested into the cell. It is clear that the RGD attached to SPG increases ingestion as presented by (d). When importin-β was attached to SPG (e), the complex is more localized in the nucleus than the others, indicating that importin-β induced nuclear transport after the complex was ingested into the cytosol. Our visual observation revealed that approximately 10% of the cells contained the green-stained nucleus, that is, it contained FITC-poly$(dA)_{40}$.

Conclusion

A new strategy to deliver ODN to nucleus by use of a novel complex made from SPG and ODNs was developed. Nuclear transport is strictly regulated by the nuclear pore size and related proteins, and the pour size is about 50 nm in diameter. The smaller molecular weight of SPG makes nuclear transport easier; however, the complex stability decreases with a decrease in the molecular weight. We found that the best molecular weight was 25 K. We attached importin-β to the side chain of SPG by simply mixing biotin-attached SPG and

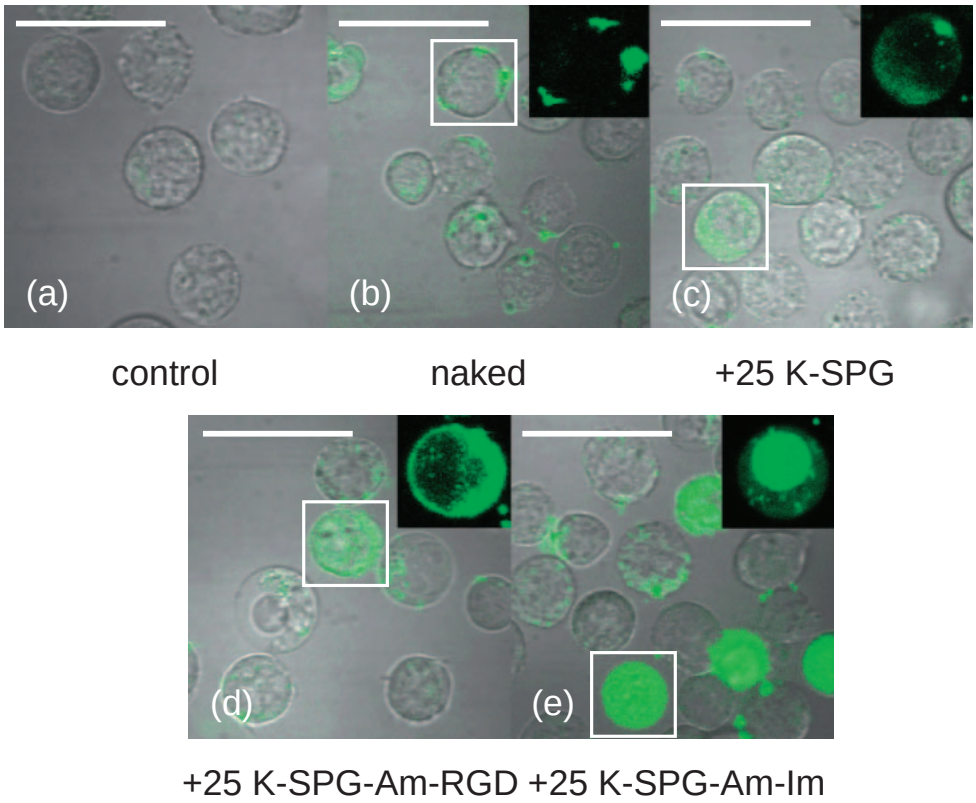

Fig. 6. Fluorescence microscopy to show the intracellular distribution of SPG/FITC-poly(dA)$_{40}$ (net FITC-poly(dA)$_{40}$ was 5 μM) after cationic lipid (Lipofectamine™, 2 μL) mediated uptake for Jurkat cells (0.5×10^6 cells mL^{-1}, 5 h). (a): Jurkat cell control, (b): naked FITC-poly(dA)$_{40}$, (c): 25 K-SPG, (d): 25 K-SPG-Am-RGD, and (e): 25 K-SPG-Am-Im, respectively. All bars shows 5 μm.

streptavidin-fused importin-β (25 K-SPG-Am-Im). When the complex formed from 25 K-SPG-Am-Im and tAS was added into Jurkat cells, the telomerase activity was more suppressed than that of the naked dose, indicating that the importin-β in the complex induced nuclear transport of the complexed tAS and tAS causes telomerase inhibition. This new methodology for nuclear anti-sense therapy should be important in the future.

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Supporting Information

The cited data in the main text: S3, S4 and S5 are in the supplementary information, as well as the others. These materials are available free of charge on the Web at: http://www.csj.jp/journals/bcsj/.

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This is the 43rd paper in the series of "Polysaccharide-Polynucleotide Complexes."

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