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Short communication

Preparation of fluoroalkyl end-capped cooligomers/silica nanoparticles: A new approach to fluorinated nanoparticle inhibitors of Human Immunodeficiency Virus Type 1 and Simian Immunodeficiency Virus (SIV_{mac})

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Abstract

Fluoroalkyl end-capped acrylic acid and sulfonic acid cooligomers reacted with tetraethoxysilane (TEOS) and silica/nanoparticles under alkaline conditions to afford the corresponding cooligomers/silica nanoparticles (mean diameters: 32–173 nm) with a good dispersibility and stability in aqueous and organic media. Interestingly, fluorinated nanoparticles containing carboxy groups were found to exhibit a potent and selective anti-HIV-1 activity in vitro. In contrast, fluorinated cooligomers containing sulfo groups were shown to have a potent and selective anti-SIV_{mac} activity in vitro.

Keywords: Fluorinated oligomer; Silica nanoparticle; Hybrid; Anti-HIV-1 activity; Anti-SIV_{mac} activity

1. Introduction

It is well known that fluorinated polymers such as poly(tetrafluoroethylene) and poly(chlorotrifluoroethylene/vinylether) are functional materials due to exhibiting excellent chemical and thermal stability, low surface energy, and low refractive index and dielectric constant [1]. In these fluorinated polymers, partially fluorinated, in particular fluoroalkyl end-capped oligomers are attractive materials, because they exhibit various unique properties such as high solubility, surface active properties, and nanometer size-controlled self-assembled molecular aggregates which cannot be achieved by the corresponding non-fluorinated, randomly or block-type fluoroalkylated polymers, and low-molecular weight fluorinated surfactants [2]. For example, fluoroalkyl end-capped acrylic acid-trimethylvinylsilane cooligomers can form the nanometer size-controlled self-assembled molecular aggregates, and these fluorinated molecular aggregates could interact with Human Immunodeficiency Virus Type 1 (HIV-1) as a guest molecule to exhibit a potent and selective anti-HIV-1 activity in vitro [3]. In general, the shape of these fluorinated molecular aggregates is easily exchangeable under a variety of conditions. Thus, it is in particular interest to prepare new fluoroalkyl end-capped oligomer-coated nanoparticles possessing anti-HIV-1 activity in which their structures are fixed from the viewpoint of the development of new fluorinated functional materials.

In this communication, we would like to demonstrate on the preparation, and anti-HIV-1 and anti-Simian Immunodeficiency Virus (SIV_{mac}) activities of new fluoroalkyl end-capped cooligomers/silica nanoparticles.

2. Results and discussion

Firstly, we tried to study the inhibitory effects on the replication of HIV-1 and SIV_{mac} in cell cultures of fluoroalkyl end-capped trimethylvinylsilane–acrylic acid cooligomers {R_F-$(CH_2CHSiMe)_x$-$(CH_2CHCOOH)_y$-R_F [R_F-(VM-Si)$_x$-(ACA)$_y$-R_F]; $R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$; $x{:}y = 5{:}95$; Mn = 5030} [4] and fluoroalkyl end-capped dimethylacrylamide-2-methacryloyloxyethanesulfonic acid cooligomer

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Table 1
Inhibitory effects of R_F-cooligomers on the replication of HIV-1 and SIV_{mac} in cell cultures

R_F-cooligomer	Virus	Cell	EC_{50} (μg/ml)[a]	CC_{50} (μg/ml)[b]
R_F-(VM-Si)$_x$-(ACA)$_y$-R_F	SIV_{mac}	M8166	69 ± 10	>100
	HIV-1	M8166	31 ± 12	>100
	HIV-1	MT-4	2.2 ± 0.90	>100
R_F-(DMAA)$_x$-(MES)$_y$-R_F	SIV_{mac}	M8166	0.081 ± 0.016	>100
	HIV-1	M8166	>100	>100
	HIV-1	MT-4	9.4 ± 4.3	>100

[a] 50% effective concentration.
[b] 50% cytotoxic concentration.

{R_F-($CH_2CHCONMe_2$)$_x$-($CH_2CMeCO_2CH_2CH_2SO_3H$)$_y$-R_F [R_F-(DMAA)$_x$-(MES)$_y$-R_F]; R_F = $CF(CF_3)OC_3F_7$; *x*:*y* = 59:41; Mn = 16,600} [4], and these results were shown in Table 1.

As shown in Table 1, R_F-(VM-Si)$_x$-(ACA)$_y$-R_F cooligomers were found to exhibit a potent and selective anti-SIV_{mac} and anti-HIV-1 activities for M8166 cells and MT-4 cells, because this cooligomer shows the lower EC_{50} values (from 2.2 to 69 μmg/ml), in contrast CC_{50} value is greater than 100 μg/ml in each case. On the other hand, R_F-(DMAA)$_x$-(MES)$_y$-R_F cooligomer shows a higher potent and selective anti-SIV_{mac} activity for M8166 cells. However, unexpectedly, this cooligomer was not able to exhibit anti-HIV-1 activity for M8166 cells.

Next, we tried to prepare novel R_F-(VM-Si)$_x$-(ACA)$_y$-R_F/silica nanoparticles and R_F-(DMAA)$_x$-(MES)$_y$-R_F/silica nanoparticles by the reactions of the corresponding cooligomers with tetraethoxysilane (TEOS) and silica nanoparticles under alkaline conditions. These results were shown in Scheme 1 and Table 2.

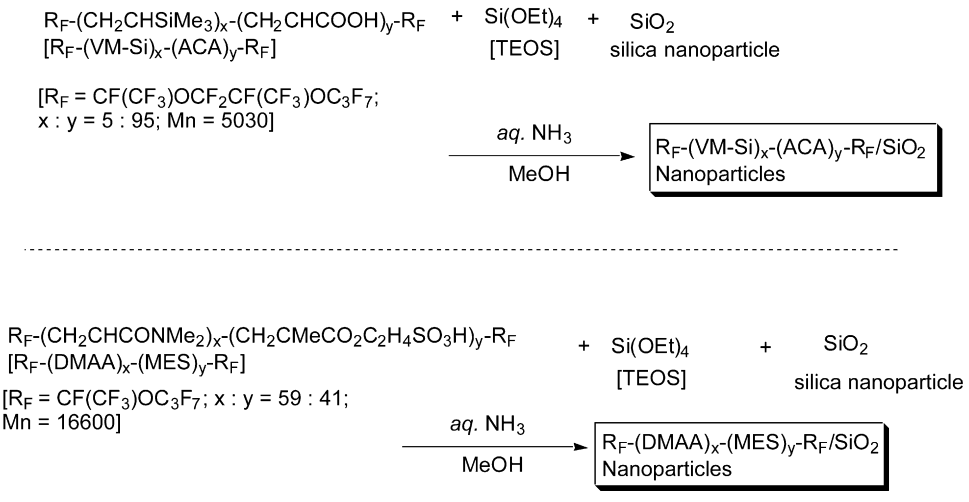
As shown in Scheme 1 and Table 2, a variety of fluoroalkyl end-capped cooligomers/silica nanoparticles were obtained in 39–60% isolated yields. We have measured the size of R_F-(VM-Si)$_x$-(ACA)$_y$-R_F and R_F-(DMAA)$_x$-(MES)$_y$-R_F cooligomers/silica nanoparticles in methanol by the use of dynamic light-scattering (DLS) measurements at 30 °C. Interestingly, the size of the obtained fluorinated nanoparticles was found to increase from 32 nm (or 37 nm) to 173 nm (or 141 nm) with the increase of the size of the used silica nanoparticles from 11 to 95 nm. This finding suggests that the hybridization of fluoroalkyl end-capped cooligomers with TEOS and silica nanoparticles should be smoothly proceeded under mild conditions.

Interestingly, a variety of isolated fluorinated cooligomers/silica nanoparticle powders were found to exhibit a superior redispersibility and stability in methanol. The size of the redispersed fluorinated particles did not change even after the redispersion of the parent fluorinated particle powders into methanol (see Table 3).

We have studied the anti-HIV-1 activity and anti-SIV_{mac} activity of R_F-(VM-Si)$_x$-(ACA)$_y$-R_F and R_F-(DMAA)$_x$-(MES)$_y$-R_F cooligomers/silica nanoparticles, and the results were shown in this Table 4.

As shown in Table 4, R_F-(VM-Si)$_x$-(ACA)$_y$-R_F cooligomer/silica nanoparticles were shown to exhibit a potent and selective anti-HIV-1 activity for M8166 cells and MT-4 cells, because EC_{50} values are small and CC_{50} value are greater than 100 μg/ml in each case, although this nanoparticle could not exhibit anti-SIV_{mac} activity. On the other hand, R_F-(DMAA)$_x$-(MES)$_y$-R_F cooligomer/silica nanoparticle was found to exhibit a good anti-SIV_{mac} activity; however this nanoparticle failed to exhibit anti-HIV-1 activity for both M8166 cells and MT-4 cells.

Thus, we tried to study the relationship between the size of fluorinated cooligomer/silica nanoparticles and anti-HIV-1 activity including anti-SIV_{mac} activity, and the results were shown in Table 5.



Scheme 1.

Table 2
Preparation of R_F-(DMAA)$_x$-(MES)$_y$-R_F/SiO_2 and R_F-(VM-Si)$_x$-(ACA)$_y$-R_F/SiO_2 nanoparticles

Rp-cooligomer	TEOS (g)	30% SiO_2 nanoparticle MeOH solution [g [size of particle]]	R_F-cooligomer/SiO_2 nanoparticles	
			Yield[a](%)	Particle size (nm)
R_F-(DMAA)$_x$-(MES)$_y$-R_F				
0.20 g	0.19 g (0.9 mmol)	1.33 g [11 nm]	48	31.7 ± 3.8
0.20 g	0.19 g	1.33 g [45 nm]	44	67.9 ± 10.1
0.20 g	0.19 g	1.33 g [95 nm]	39	173.2 ± 32.1
R_F-(VM-Si)$_x$-(ACA)$_y$-R_F				
0.20 g	0.19 g (0.9 mmol)	1.33 g [11 nm]	60	37.2 ± 4.9
0.20 g	0.19 g	1.33 g [45 nm]	44	92.5 ± 24.0
0.20 g	0.19 g	1.33 g [95 nm]	39	141.2 ± 30.1

[a] Isolated yield based on oligomer (0.20 g), SiO_2 [(0.9 mmol) (0.05 g)], and SiO_2 nanoparticle (0.40 g).

Table 3
Size of R_F-(DMAA)$_x$-(MES)$_y$-R_F/SiO_2 and R_F-(VM-Si)$_x$-(ACA)$_y$-R_F/SiO_2 nanoparticles in methanol determined by dynamic light scattering measurements

Size of used parent SiO_2 nanoparticles (nm)	Size of dispersed particles (nm)	Size of redispersed particles (nm)
R_F-(DMAA)$_x$-(ACA)$_y$-R_F/SiO_2 nanoparticle		
11	31.7 ± 2.8	39.3 ± 4.9
45	67.9 ± 10.1	94.4 ± 21.3
95	173.2 ± 32.1	160.9 ± 26.2
R_F-(VM-Si)$_x$-(ACA)$_y$-R_F/SiO_2 nanoparticle		
11	37.2 ± 3.8	42.2 ± 4.1
45	92.5 ± 24.0	109.8 ± 23.1
95	141.2 ± 30.1	149.5 ± 28.8

As shown in Table 5, we could not obtain any good relationships between the particle size and antiviral activity. In these nanoparticles, R_F-(DMAA)$_x$-(MES)$_y$-R_F-cooligomeric nanoparticles were shown to possess a good anti-SIV_{mac} activity; however these nanoparticles have no anti-HIV-1 activity. On the other hand, R_F-(VM-Si)$_x$-(ACA)$_y$-R_F cooligomeric nanoparticles were found to have a good anti-HIV-1 activity; although these nanoparticles have no anti-SIV_{mac} activity at all.

In order to study the more detailed relationship between the anti-HIV-1 activity including anti-SIV_{mac} activity and fluorinated cooligomers/silica nanoparticles, we prepared a variety of fluorinated cooligomeric nanoparticles by the use of fluoroalkanoyl peroxide as a key intermediate as shown in this Scheme 2.

As shown in Scheme 2 and Table 6, the expected fluorinated homo- and co-oligomers/silica nanoparticles were obtained from 2 to 8% isolated yields.

As shown in Table 7, DLS measurements at 30 °C show that the obtained fluorinated cooligomers/silica particles are nanometer-size controlled. These fluorinated nanoparticles were also shown to possess a good dispersibility and redisperibility in methanol and water, and the size of the redispersed nanoparticles were almost the same as that of the parent fluorinated cooligomeic nanoparticles.

We have studied on the anti-HIV-1 activity and anti-SIV_{mac} activity of these fluorinated nanoparticles thus obtained. Unfortunately, these fluorinated nanoparticles were not able to exhibit anti-HIV- 1 activity and anti-SIV activity at all (data not shown).

In this way, the size of our prepared R_F-(DMAA)$_x$-(MES)$_y$-R_F cooligomers/silica nanoparticles as shown in Tables 2 and 7 are from 32 to 409 nm, and these fluorinated nanoparticles had no anti-HIV-1 activity at all. Similarly, shorter (32 nm) or longer size (409 nm) R_F-(DMAA)$_x$-(MES)$_y$-R_F cooligomer/silica nanoparticles also could not exhibit a good anti-SIV_{mac} activity; however, R_F-(DMAA)$_x$-(MES)$_y$-R_F cooligomers/silica nanoparticles with moderate size (from 68 to 173 nm) were found to exhibit a higher anti-SIV_{mac} activity (see Table 5). This would be due to the size fitness between the size of SIV and fluorinated particles.

In summary, R_F-(VM-Si)$_x$-(ACA)$_y$-R_F/silica nanoparticles have a potent and selective anti-HIV-1 activity. In contrast, R_F-(DMAA)$_x$-(MES)$_y$-R_F/silica nanoparticles were found to

Table 4
Inhibitory effects of R_F-cooligomer/SiO_2 nanoparticles on the replication of HIV-1 and SIV_{mac} in cell cultures

Rp-cooligomer	Virus	Cell	EC_{50} (μg/ml)[a]	CC_{50} (μg/ml)[b]
R_F-(ACA)$_x$-(VM-Si)$_y$-R_F/SiO_2 nanoparticles (particle size: 180 nm)	SIV_{mac}	M8166	>100	>100
	HIV-1	M8166	100	>100
	HIV-1	MT-4	8.1 ± 4.6	>100
R_F-(DMAA)$_x$-(MES)$_y$-R_F/SiO_2 nanoparticles (particle size: 126 nm)	SIV_{mac}	M8166	73 ± 18	>100
	HIV-1	M8166	>100	>100
	HIV-1	MT-4	>100	>100

[a] 50% effective concentration.
[b] 50% cytotoxic concentration.

Table 5
Relationship between the size of R_F-cooligomer/SiO_2 nanoparticles and antiviral activity

Size of nanoparticles (nm)	HIV-1/MT-4		SIV_{mac}/M8166	
	EC_{50} (μg/ml)	CC_{50} (μg/ml)	EC_{50} (μg/ml)	CC_{50} (μg/ml)
R_F-$(DMAA)_x$-$(MES)_y$-R_F/SiO_2 nanoparticle				
32	>100	>100	100	>100
68	>100	>100	35 ± 17	>100
126	>100	>100	73 ± 18	>100
173	>100	>100	33 ± 14	>100
R_F-$(VM\text{-}Si)_x$-$(ACA)_y$-R_F/SiO_2 nanoparticle				
37	8.4 ± 4.9	>100	>100	>100
93	6.9 ± 4.4	>100	>100	>100
141	8.4 ± 3.4	>100	>100	>100
180	8.1 ± 4.6	>100	>100	>100

Table 6
Preparation of fluorinated homo- and co-oligomers/SiO_2 nanoparticles by the use of fluoroalkanoyl peroxide

$(R_FCOO)_2$[a] (mmol)	MESor ACA (mmol)	DMAA or ACMO (mmol)	SiO_2 nanoparticles (g)	R_F-oligomers/SiO_2 nanoparticle yield[b] (%)
3.1	MES (13.6)	–	0.50	R_F-$(MES)_n$-R_F/SiO_2 (7)
2.7	MES (13.6)	DMAA (13.8)	0.50	R_F-$(DMAA)_x$-$(MES)_y$-R_F/SiO_2 (6)
2.9	MES (13.7)	ACMO (13.6)	0.50	R_F-$(ACMO)_x$-$(MES)_y$-R_F/SiO_2 (8)
2.7	ACA (13.9)	DMAA (13.6)	0.50	R_F-$(DMAA)_x$-$(ACA)_y$-R_F/SiO_2 (2)

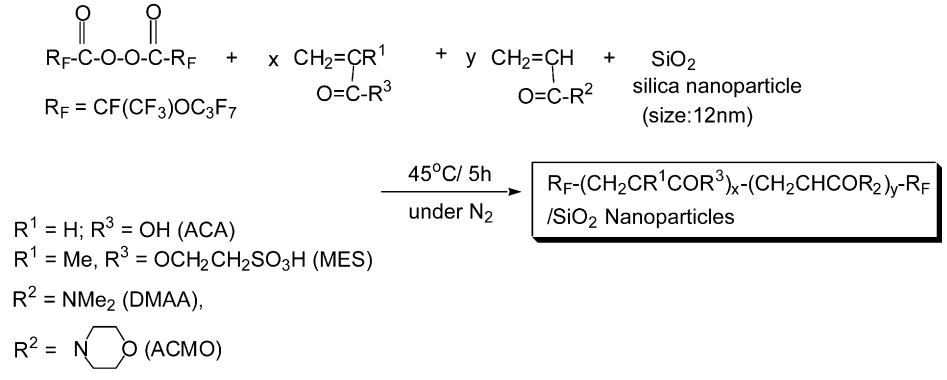
[a] $R = CF(CF_3)OC_3F_7$.
[b] Isolated yield based on the decarboxylated peroxide unit (R_F-R_F), MES (or ACA), DMAA(or ACMO), and SiO_2 nanoparticle (0.50 g).

exhibit a potent and selective anti-SIV_{mac} activity. The original R_F-$(VM\text{-}Si)_x$-$(ACA)_y$-R_F and R_F-$(DMAA)_x$-$(MES)_y$-R_F have higher anti-HIV-1 activity (EC_{50} = 2.2 μg/ml) and anti-SIV_{mac} activity (EC_{50} = 0.081 μg/ml), respectively. However, the contents of R_F-$(VM\text{-}Si)_x$-$(ACA)_y$-R_F in nanoparticles were estimated to be 16% (particle size: 37 nm in Table 5), 17% (particle size: 141 nm in Table 5) and 21% (particle size: 93 nm in Table 5) by thermogravimetric analyses. Similarly, the contents of R_F-$(DMAA)_x$-$(MES)_y$-R_F cooligomers/silica nanoparticles were estimated to be 6% (particle size: 173 nm in Table 5) and 10% (particle size: 68 nm in Table 5) by the use of elemental analyses of fluorine. Therefore, it was verified that our present fluorinated nanoparticles, especially R_F-$(VM\text{-}Si)_x$-$(ACA)_y$-R_F/silica nanoparticles are more active for the inhibitory effect for HIV-1 replication compared to that of the parent R_F-$(VM\text{-}Si)_x$-$(ACA)_y$-R_F cooligomer. Our present fluoroalkyl end-capped cooligomeric silica nanoparticles are expected to be widely applicable not only for antiviral polymeric drugs but also the fields of the materials science. Further studies are actively in progress.

3. Experimental

3.1. Preparation of R_F-$(VM\text{-}Si)_x$-$(ACA)_y$-R_F/silica nanoparicles

To a methanol solution (10 ml) of R_F-$(VM\text{-}Si)_x$-$(ACA)_y$-R_F (0.20 g)] were added tetraethoxysilane (TEOS: 0.19 g), silica nanoparticle methanol solution [30% (wt.): 1.33 g; average particle size: 11 nm (methanol silica-sol (TR): Nissan Chemical Industrials Ltd., Tokyo, Japan)], and 25% aqueous ammonia solution (0.20 ml). The mixture was stirred with a



Scheme 2.

Table 7
Size of fluorinated homo- and co-oligomers/SiO_2 nanoparticles, which were prepared by the use of fluoroalkanoyl peroxide, in methanol and aqueous solutions

R_F-oligomer/SiO_2 nanoparticles	Size of dispersed particles (nm)	Size of redispersed particles (nm)
R_F-$(MES)_n$-R_F/SiO_2[a]	32.6 ± 3.3	35.4 ± 2.2
R_F-$(DMAA)_x$-$(MES)_y$-R_F/SiO_2[a]	408.9 ± 46.8	425.7 ± 43.4
R_F-$(ACMO)_x$-$(MES)_y$-R_F/SiO_2[b]	36.5 ± 3.3	55.7 ± 5.7
R_F-$(DMAA)_x$-$(ACA)_y$-R_F/SiO_2[a]	52.0 ± 9.6	56.6 ± 5.9

[a] Methanol solution.
[b] Aqueous solution.

magnetic stirring bar at room temperature for 2 h. After the solvent was evaporated off, to the obtained crude products was added methanol (10 ml). The methanol solution was stirred with magnetic stirring bar at room temperature for 2 day, and then was centrifuged for 30 min. The expected fluorinated nanocomposite was easily separated from the methanol solution. Fluorinated nanocomposite powders thus obtained were dried in vacuo at 50 °C for 2 day to afford purified particle powders (0.39 g). R_F-$(DMAA)_x$-$(MES)_y$-R_F nanocomposites were also prepared under similar conditions.

3.2. Preparation of R_F-$(MES)_n$-R_F/silica nanoparticles by the use of fluoroalkanoyl peroxide

Perfluoro-2-methyl-3-oxahexanoyl peroxide (3.1 mmol) in 1:1 mixed solvents (AK-225) of 1,1-dichloro-2,2,3,3,3-pentafluoropropane and 1,3-dichloro-1,2,2,3,3-pentafluoropropane (120 g) was added to MES (13.6 mmol) and silica nanoparticle aqueous solution [20 wt. %: 2.50 g; average particle size: 11 nm (Nissan Chemical Industrials Ltd., Tokyo, Japan)]. The heterogeneous solution was stirred at 45 °C for 5 h under nitrogen. After the solvent was evaporated off, to the obtained crude products was added methanol (25 ml). The methanol solution was stirred with magnetic stirring bar at room temperature for 1 day, and then was centrifuged for 30 min. The expected fluorinated nanoparticles were easily separated from the methanol solution. These fluorinated nanoparticles were also dispersed into methanol, and then was centrifuged for 30 min. Fluorinated nanoparticle powders thus obtained were dried in vacuo at 50 °C for 2 day to afford purified particle powders (0.35 g). Other fluorinated cooligomer/silica nanoparticles were prepared by the use of fluoroalkanoyl peroxide under similar conditions.

3.3. Antiviral assays

Antiviral activity of the compounds against HIV-1 (HTLB-IIIb strain) [5] and SIV_{mac} (mac251 strain) [6] replication was based on the inhibition of the virus-induced cytopathic effect in MT-4 cells [7] and M8166 cells [8], respectively, as described previously [7].

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