P20-04

Effect of Chemical Modification of 5'-End of siRNA on the Recognition of Asymmetry and the Strand Selection

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Abstract

In the present study, the efficiency of RNA interference (RNAi) of small interfering RNAs (siRNAs) bearing 5'-O-methyl-2'-deoxythymidine (X) and 5'-amino-2', 5'-dideoxythymidine (Z) at the 5'-end of the sense strand and the antisense strand of siRNA was investigated in HeLa cells stably expressing EGFP. The results indicated that when one strand of siRNA was modified with X or Z and the other was unmodified, the X or Z modification was predominant in the process of strand selection and the unmodified strand was selected as a guide strand. When both strands are modified with X or Z, the modified antisense strand with X or Z will be selected as a guide strand with a certain probability. The resulting mature RISC exerted reduced but still some silencing activity remained. These results suggest that the modification of the sense strand with X or Z eliminates the off-target effects caused by the sense strand without affecting the silencing efficiency of the siRNA.

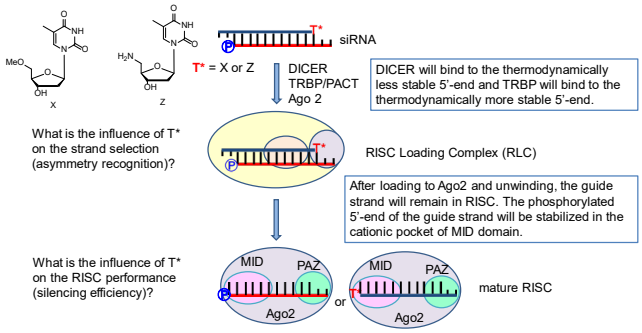

Figure 1. Effect of Chemical Modification of 5'-End of siRNA on RNA Interference

Table 1. Component of siRNA1-siRNA16

sense (s): 5'-R¹ACGGCAAGCUGACCCUGA(Am)(Gm)-3'
antisense (as): 5'-R²CAGGGUCAGCUUGCCGUA(Gm)(Gm)-3'

		R¹			
		U	T	X	Z
R²	U	siRNA1	siRNA5	siRNA9	siRNA13
	T	siRNA2	siRNA6	siRNA10	siRNA14
	X	siRNA3	siRNA7	siRNA11	siRNA15
	Z	siRNA4	siRNA8	siRNA12	siRNA16

A, G, C, U = RNA, T = 2'-deoxythymidine, (Nm) = 2'-OMeRNA,
X = 5'-O-methyl-2'-deoxythymidine, Z = 5'-amino-2',5'-dideoxythymidine

Table 2. Thermodynamic Parameters of 5'-Ends of siRNA1

S: 3'-gaAGUCCCAGUCGAACGGCAU-5'
AS: 5'-UCAGGGUCAGCUUGCCGUAgg-3'

AG/UC + GU/CA ΔΔG°37 = - 0.9 (kcal mol-1) CA/GU + AU/UA

ΔH° (kcal mol-1)	-13.3 + (-10.5) = -23.8	-10.2 + (-8.1) = -18.3
ΔS°(kcal mol-1 K-1)	-35.5 + (-27.8) = -63.3	-26.2 + (-22.6) = -48.8
ΔG°37(kcal mol-1)	-2.3 + (-1.8) = **-4.1**	-2.1 + (-1.1) = **-3.2**

N. Sugimoto, et al, Biochemistry, 34, 11211 (1995).

siRNA1 was designed to have U at the 5'-end of the antisense strand and the sense strand and to have almost the same thermodynamic stabilities of the three base pairs of the two ends.

Table 3. Thermodynamic Parameters of 5'-Ends of siRNA2-4

S: 3'-gaAGUCCCAGUCGAACGGCAU-5'
AS: 5'-TCAGGGUCAGCUUGCCGUAgg-3'

AG/TdC + GU/CA ΔΔG°37 = + 0.1 (kcal mol-1) (cf. -1.0 for UC) CA/GU + AU/UA

ΔH° (kcal mol-1)	-5.5 *+ (-10.5) = -16.0	-10.2 + (-8.1) = -18.3
ΔS°(kcal mol-1 K-1)	-13.5* + (-27.8) = -41.3	-26.2 + (-22.6) = -48.8
ΔG°37(kcal mol-1)	-1.3* + (-1.8) = **-3.1** (cf. -4.1 for UC)	-2.1 + (-1.1) = **-3.2**

*Parameters for RNA/DNA were used.
N. Sugimoto, et al, Biochemistry, 34, 11211 (1995).

Table 4. Thermodynamic Parameters of 5'-Ends of siRNA5

S: 3'-gaAGUCCCAGUCGAACGGCAT-5'
AS: 5'-UCAGGGUCAGCUUGCCGUAgg-3'

AG/UC + GU/CA ΔΔG°37 = -1.4 (kcal mol-1) CA/GU + dAT/UA

ΔH° (kcal mol-1)	-13.3 + (-10.5) = -23.8	-10.2 + (-7.8) *= -18.0
ΔS°(kcal mol-1 K-1)	-35.5 + (-27.8) = -63.3	-26.2 + (-23.2)* = -49.4
ΔG°37(kcal mol-1)	-2.3 + (-1.8) = **-4.1**	-2.1 + (-0.6)* = **-2.7**

*Parameters for RNA/DNA were used.
N. Sugimoto, et al, Biochemistry, 34, 11211 (1995).

Table 5. Thermodynamic Parameters of 5'-Ends of siRNA6

S: 3'-gaAGUCCCAGUCGAACGGCAT-5'
AS: 5'-TCAGGGUCAGCUUGCCGUAgg-3'

AG/TdC + GU/CA ΔΔG°37 = -0.4 (kcal mol-1) Cf. -1.4 for UC CA/GU + dAT/UA

ΔH° (kcal mol-1)	-5.5* + (-10.5) = -16.0	-10.2 + (-7.8)* = -18.0
ΔS°(kcal mol-1 K-1)	-13.5* + (-27.8) = -41.3	-26.2 + (-23.2)* = -49.4
ΔG°37(kcal mol-1)	-1.3* + (-1.8) = **-3.1**	-2.1 + (-0.6)* = **-2.7**

*RNA/DNAのパラメーター多使用。

The thermodynamic stabilities of the two ends of siRNA1-siRNA16 should have almost no or very little effect on the recognition of the asymmetry of siRNA and on the guide strand selection in RISC to enable pure evaluation of the effect of 5'-methoxy and 5'-amino groups on the guide strand selection and the silencing efficiency.

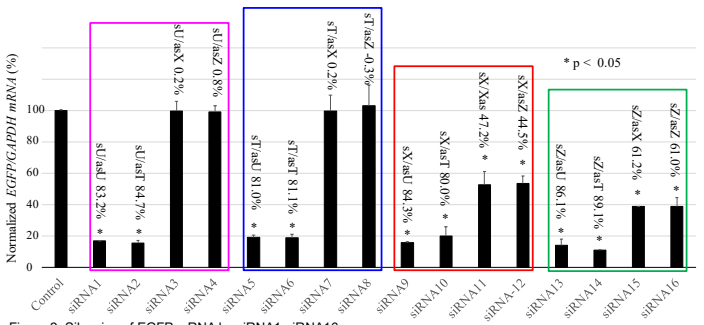

Figure 2. Silencing of EGFP mRNA by siRNA1-siRNA16.
HeLa (5 x 10^4 cells /well,10 % FBS/MEM) maintained in 5% CO_2, at 37°C, for 24h were transfected with siRNA at the final concentration of 100 nM using Lipofectamine® 2000 as described in the Materials and Methods. The values represent the mean

Silencing Efficiencies of siRNA1 (sU/asU), siRNA2 (sU/asT), siRNA3 (sU/asX), siRNA4 (sU/asZ)

The silencing efficiencies of siRNA1 (sU/asU) and siRNA2 (sU/asT) at 100 nM were 83.2% and 84.7%, respectively (FIG 4). Substitution of U at the 5'-end of the antisense strand for T had no effect on silencing efficiencies of siRNA, in other words, this change had no effect on the strand selection and the RISC activity. It should be noted that both siRNA3 (sU/asX) and siRNA4 (sU/asZ) did not show any silencing efficiency. This could be an indication of the fact that X or Z on the antisense strand had a critical effect on the strand selection during the process of RLC or RISC formation so that the sense strand with 5'-U was predominantly selected as a guide strand in RISC. In addition, it is plausible that X or Z on the antisense strand decreased RISC performance thereby abolishing silencing efficiencies of the siRNA. The silencing efficiencies of siRNA11 (sX/asX) and siRNA12 (sX/asZ) at 100 nM were 47.2% and 44.5%, respectively whereas those of siRNA15 (sZ/asX) and siRNA16 (sZ/asZ) were 61.2% and 61.0%, respectively, which could be due to the reduced activity of the RISC bearing the antisense strand modified with X or Z as a guide strand leading to decreased but still remaining silencing activity. Therefore, the effect of X and Z on the strand selection was more important than that on RISC performance.

Silencing Efficiencies of siRNA5 (sT/asU), siRNA6 (sT/asT), siRNA7 (sT/asX), siRNA8 (sT/asZ)

The silencing efficiencies of siRNA5 (sT/asU) and siRNA6 (sT/asT) at 100 nM were 81.0% and 81.1%, respectively. Similar to siRNA1 (sU/asU) and siRNA2 (sU/asT), substitution of U at the 5'-end of the antisense strand for T had no effect on the silencing efficiencies of these siRNAs. The silencing efficiencies of siRNA7 (sT/asX) and siRNA8 (sT/asZ) at 100 nM were only 0.2% and -0.3%, respectively. These results are consistent with the perception for siRNA3 (sU/asX) and siRNA4 (sU/asZ) that X or Z on the antisense strand had a critical effect on the strand selection during the process of RLC and RISC formation so that the sense strand with 5'-T was predominantly selected as a guide strand in RISC.

Silencing Efficiencies of siRNA9 (sX/asU), siRNA10 (sX/asT), siRNA11 (sX/asX), siRNA12 (sX/asZ)

Silencing efficiencies of siRNA9 (sX/asU) and siRNA10 (sX/asT) at 100 nM were 84.3% and 80.0%, respectively, which was similar to those of siRNA1 (sU/asU) and siRNA2 (sU/asT). As described above, it was expected that 5'-methoxy group of the sense strand of siRNA9 (sX/asU) and siRNA10 (sX/asT) would increase the probability for the antisense strand with 5'-U or T to be selected as a guide strand and result in higher silencing efficiency. But the improvement of the silencing efficiencies of siRNA9 (sX/asU) and siRNA10 (sX/asT) was not significant compared with those of siRNA1 (sU/asU) and siRNA2 (sU/asT). The silencing efficiencies of siRNA11 (sX/asX) and siRNA12 (sX/asZ) were 47.2% and 44.5%, respectively. These results indicate that modification of the 5'-end of the sense strand with X increased the probability for the antisense strand with 5'-X or Z to be selected as a guide strand in RISC and that the RISC bearing the antisense strand with 5'-X or Z as a guide strand retained moderate silencing ability.

Silencing Efficiencies of siRNA13 (sZ/asU), siRNA14 (sZ/asT), siRNA15 (sZ/asX), siRNA16 (sZ/asZ)

The silencing efficiencies of siRNA15 (sZ/asX) and siRNA16 (sZ/asZ) at 100 nM were 61.2% and 61.0%, respectively. As discussed above, the sense strand with 5'-Z increased the probability for the antisense strand with 5'-X or Z to be selected as a guide strand in RISC and the RISC bearing the antisense strand modified with X or Z at 5'-end as a guide strand exerted reduced but still remaining silencing activity. Moreover, comparing the results of siRNA3 (sU/asX) with siRNA9 (sX/asU), siRNA4 (sU/asZ) with siRNA13 (sZ/asU), siRNA7 (sT/asX) with siRNA10 (sX/asT) and siRNA8 (sT/asZ) with siRNA14 (sZ/asT), respectively, it was clearly indicated that X or Z at 5'-end of one strand displayed determinant effect on the recognition of asymmetry of siRNA and dramatically increased the probability for the other strand to be selected as a guide strand. Therefore, it can be concluded that the effect of X and Z on the strand selection during RLC and RISC formation is more significant than that on the RISC performance.

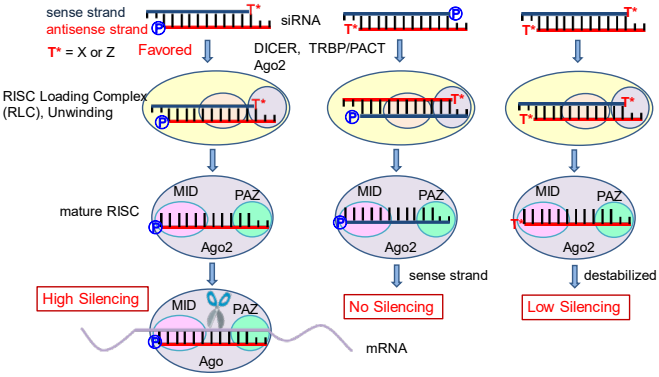

Figure 3. Influence of Modification of siRNA with X and Z on RNAi Processes.

Conclusions

When one strand of the siRNA is modified with X or Z and the other is unmodified, the X or Z modification will be predominant in the process of strand selection and the unmodified strand will be designated as a guide strand. The results that siRNA3, siRNA4, siRNA7 and siRNA8 did not exhibit any silencing activity indicated that the unmodified sense strand was predominantly selected as a guide strand. These results can be ascribed to the influence of the modification in a double stranded state.

When both strands are modified with X or Z, the antisense strand modified with X or Z will be selected as a guide strand with a certain probability. The resulting mature RISC exerted reduced but retained some silencing activity as observed for siRNA11, siRNA12, siRNA15 and siRNA16. The reduced silencing activities of siRNA11, siRNA12, siRNA15 and siRNA16 compared with siRNA1 and siRNA2 can be mainly ascribed to the influence of the modification in a single stranded state.

To conclude, siRNA bearing a sense strand modified with X or Z and an unmodified antisense strand will eliminate the disfavored silencing activity (off-target effect) caused by the sense strand without affecting the silencing activity induced by the antisense strand (**Figure 3**).

COI: We have no conflict of interest to disclose for this presentation.