[S4-2]

Substrate Structural Requirement for Erm Protein, 23S rRNA Methyltransferase

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Erm proteins mediate one or two consecutive methylation reaction to produce the N^6-mono or dimethyl adenine of specific nucleotide (A2085 in *Bacillus subtilis* coordinate) to reduce the affinity of MLS (macrolide-lincosamide-streptogramin B) antibiotics, thereby microorganisms escape from antibiotics' action [1, 2]. According to our results and the other reports [3-5], stem 73 of 23S rRNA provides the essential structural requirement for Erm activity. Stem 73 exhibits the characteristic secondary structures including some bulge structures which are thought to be important for substrate activity in addition to some of primary structure of stem73 (Fig. 1). As base pairs comprising stem73 were truncated one base pair each time with monitoring its substrate activity, substrate activity decreased gradually until the three base pairs removed, representing 66nt substrate. Further truncation of one more base pair from 66nt RNA drastically reduced the substrate activity to be about one tenth relative to 66nt substrate (Table 1). To investigate the structural changes caused by truncation from 66nt to 64nt, structural probing with RNAse V1, A, S1 and T1 was performed. The original base pairings were disrupted by sliding 5' side of nucleotides out of base pairing and new base paring started forming A2085: U2642 in *Bacillus subtilis* coordinate and continued until base pair G2080:C2647 without constructing any bulge but with the methylatable adenine base-paired to U2642 as above and then single stranded region followed up to the G2080:C2647 (Fig. 2). So, Bulges formed by C2082, U2640 and A2641 which known to be important in substrate activity disappeared (Fig. 2). To find out how the structural changes affect the enzyme activity, kinetic study with Kd measurement of two substrates were performed to get the kinetic parameters and Kd as in Table 1. In 64nt, Kd and Km value were slightly lowered compared to 66nt, suggesting that 64nt binds to enzyme more tightly than 66nt and could be recruited easily to be methylated RNA but after being methylated, 66nt underwent the conformational change easily to be released from the enzyme faster than 64nt to produce the product 10 times more for a fixed time. Our structural probing suggested

that methylatable adenine (A2085) might be base paired with U2642 to shield the N^6 of adenine from being methylated but Erm protein have been found to adjust the conformation of methylatable adenine to be methylated [6]. It probably affects Vmax of that reaction by slowing the methylation reaction but it has proved that it is not the case.

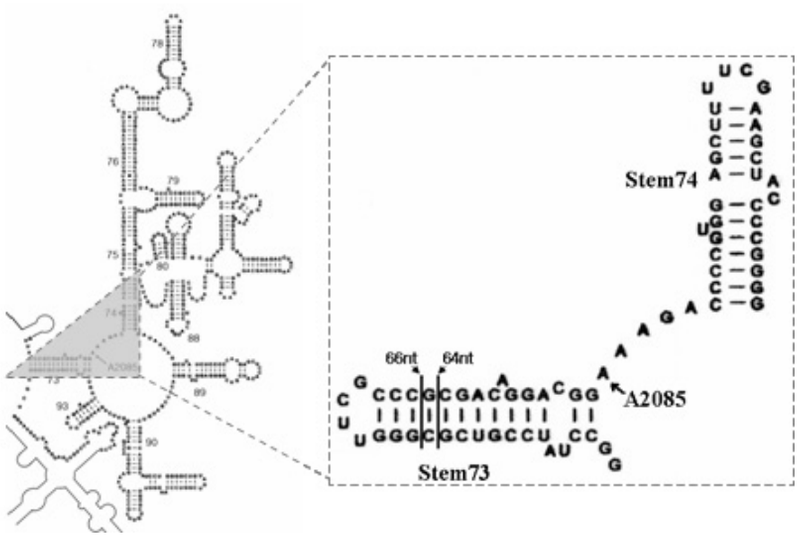

Fig. 1. Schematic secondary structure of 23S rRNA domain V and stem73-74.

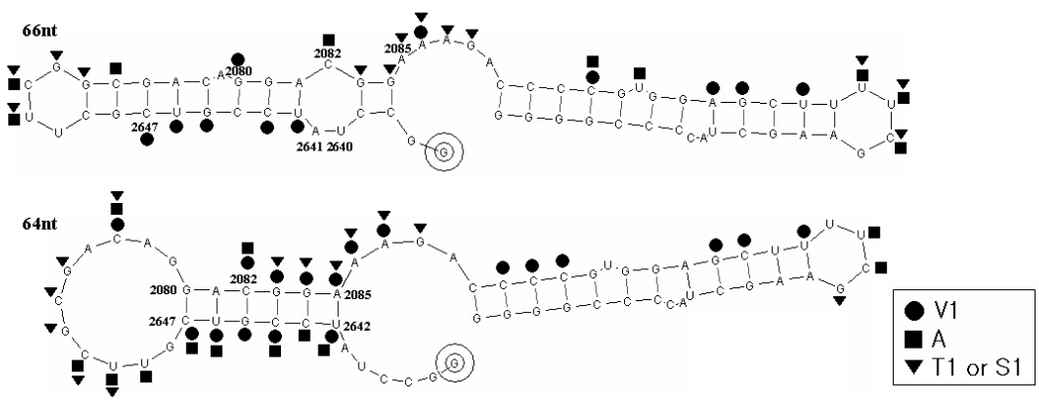

Fig. 2. 66, 64nt secondary structure probing.

Table 1. 66, 64nt activity, Kd and kinetic study

RNA	Activity (cpm)	Kd (nM)	Km (nM)	Vmax (pmol/min/mg)
66nt	20759	42±8.1	78±1.8	23.7±0.5
64nt	2107	30.2±6.7	65.3±4.3	2.7±0.2

Furthermore, unlike other Erm proteins, ErmSF contains extra long N-terminal end region 30% of amino acids of which are arginine (arginine rich motif). How the binding of arginine rich motif to 23S rRNA occurs and part of its specific binding affects the enzyme activity will be also discussed.

References

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