Structural investigations into physiological DNA phosphorothioate modification

Wenxian Lan[1], Zhongpei Hu[1], Jie Shen[1, 2], Chunxi Wang[1], Feng Jiang[3], Huili Liu[4], Dewu Long[3], Maili Liu[4], Chunyang Cao[1]*

Supplemental material and methods

PT modified DNA isolation and P-S bond stereochemistry characterization

The R_p- and S_p- PT modified single-stranded (ss) DNA were successfully isolated from their racemic mixtures by running a Proteomix SAX-NP5 strong anion-exchange column on a HPLC system. The two isolated fractions were collected and enriched so that they were enough to make NMR samples, and desalted by running ODS-C18 reverse phase column on a HPLC system. As reported in the previous literature [1], the R_p-PT ssDNA strand generally has a shorter retention time than S_p-PT DNA strand upon running SAX anion-exchange column. To characterize the stereochemistry of P-S bond in the isolated strands, the DNA hydrolysis was then performed with snake venom phosphodiesterase and alkaline phosphatase [1, 2]. The snake venom phosphodiesterase selectively digests the R_p but not S_p configuration [3]. Therefore, in our case, either the R_p-PT modified ssDNA 5'-$C_1G_2^{PS}G_3C_4C_5G_6C_7C_8G_9A_{10}$-3' or its complementary R_p-PT modified ssDNA 5'-$T_{11}C_{12}G_{13}G_{14}C_{15}G_{16}^{PS}G_{17}C_{18}C_{19}G_{20}$-3' (both with a shorter retention time than their S_p-PT strands in supplementary Figure S1) was digested into three peaks (G, C, A or T) (supplementary Figure S2), while both the S_p-PT modified ssDNA 5'-$C_1G_2^{PS}G_3C_4C_5G_6C_7C_8G_9A_{10}$-3' and its complementary S_p-PT modified ssDNA 5'-

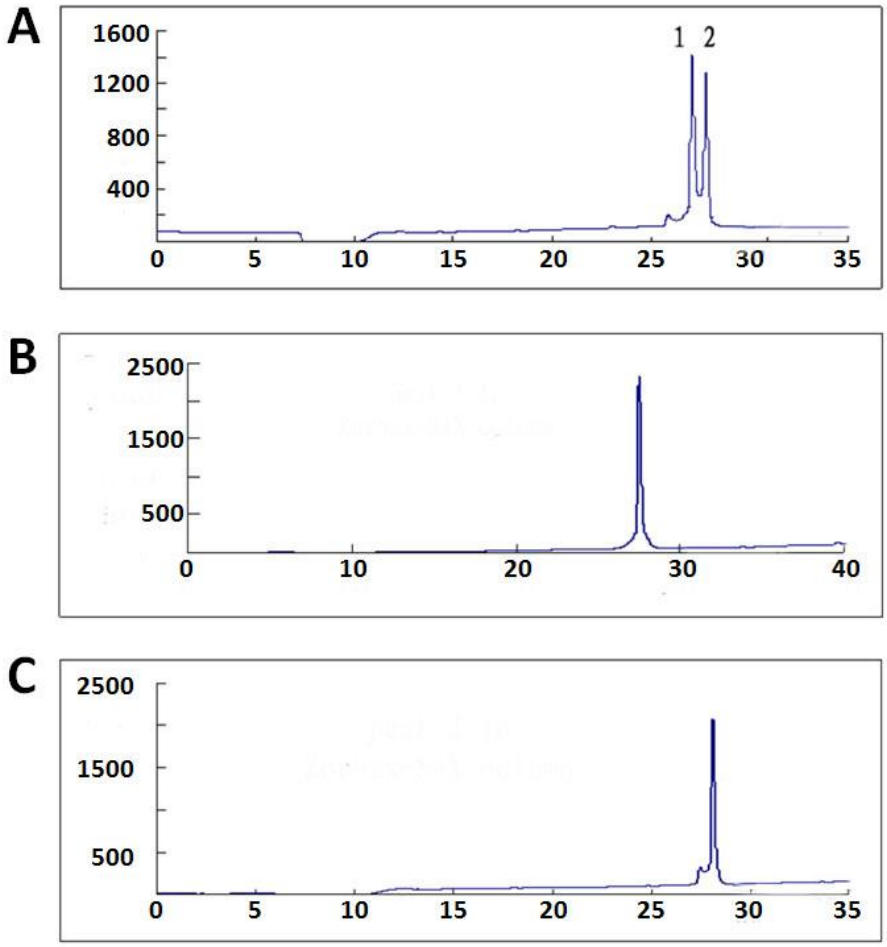
$T_{11}C_{12}G_{13}G_{14}C_{15}G_{16}{}^{PS}G_{17}C_{18}C_{19}G_{20}$-3' were digested into four peaks (G, C, -G^{PS}G-, A or T) (supplemental Figure S2).

Supplemental figures and tables

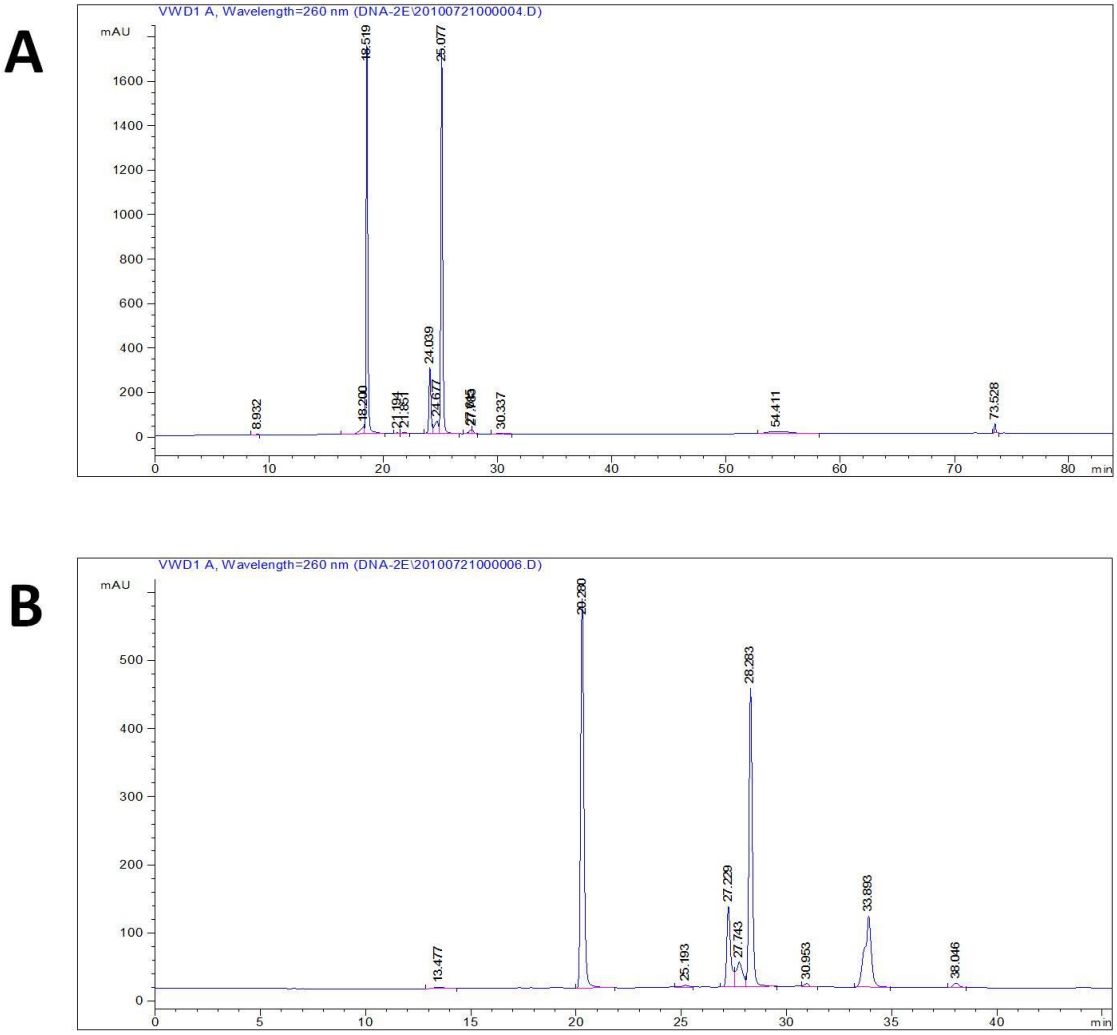
Supplemental Table S1 The electron transfer potential of PT-free dsDNA, [R_p, R_p]-PT dsDNA, [S_p, S_p]-PT dsDNA and corresponding ssDNA. N.D. means 'not detectable'.

	E_{ev} (dsDNA)	E_{ev} (ssDNA)
R_p-PT	-0.62 V	-0.37 V
S_p-PT	-0.72 V	-0.43 V
PT-free	-0.28 V	N.D.

Supplemental Fig. S1 (A) The isolation of R_p-PT ssDNA (peak 1 in A, and further purification in B) and S_p-PT ssDNA (peak 2 in A, and further purification in C) by running Proteomix SAX-NP5 strong anion-exchange column two times on HPLC system, before desalting.



Supplemental Fig. S2 The characterization of the stereochemistry of PT bond in (A) R_p-PT ssDNA and (B) S_p-PT ssDNA strands firstly by DNA hydrolysis with snake venom phosphodiesterase and alkaline phosphatase, and then by running reverse phase columns on HPLC systems, respectively.



Supplemental references

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