

Studies on DNA Phosphorothioation Modification: Chances and Challenges

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Editorial

DNA phosphorothioate (PT) modification, originally developed as an artificial tool to stabilize oligodeoxynucleotides against nuclease degradation [1], was recently found to be incorporated with sulfur into DNA backbone as a novel physiological variation by the five-gene *dnd* cluster (*dndA-E*) products in a sequence and R_p stereo specific manner [2]. This PT modification causes DNA degradation (Dnd) phenotype and is widespread and quantized in bacterial genomes, working as a part of a restriction modification system [3-5]. But this modification can be specifically cleaved *in vitro* by type IV restriction endonuclease [6]. These biochemical or genetic studies only give important but limited information on DNA PT modification. To better understand the biological function and to perform more fascinating investigations on the structural mechanism of DNA PT modification, here, we described the recent research progress in DNA PT modification.

Firstly, recent research demonstrated that the DNA with PT modification could be oxidized *in vitro* by peracetic acid or hydrogen peroxide (H_2O_2) [7], which may generate two products: DNA backbone cleavage and sulfur removal, the latter leading to the regular DNA backbone recovery. By mixing PT modified DNA hosting *Salmonella enterica* cells with H_2O_2 , DNA PT modification was observed to be related to the increased resistance to the growth inhibition by H_2O_2 , but this resistance activity against H_2O_2 resulted from DNA PT modification was disrupted when the necessary Dnd genes required for DNA PT modification were inactivated. *In vivo*, the PT modified DNA is found to be more resistant to the double-strand break damage caused by H_2O_2 than regular DNA (i.e., without PT modification). Furthermore, when the bacteria were incubated with H_2O_2 , sulfur on the PT modified DNA was removed, and the DNA was subsequently switched into regular DNA. These findings suggest that DNA PT modification provides DNA with increased reducing property, which further protects the hosting bacteria against peroxide. These studies for the first time draw an outline of the fundamental biological functions of DNA PT modification in bacteria.

Secondly, as to structural mechanism about how DNA PT modification is generated, bioinformatics studies were performed early. Among five encoded proteins required for DNA PT modification in diverse bacteria, DndC or its homolog possesses ATP pyrophosphatase activity [8,9] and was predicted to have 3'-phosphoadenosine-5'-phosphosulfate (PAPS) reductase activity, whereas DndB has homology to a group of transcriptional regulators [10,11]. DndD, also known as SpfD in *Pseudomonas fluorescens* Pf0-1, has ATPase activity possibly related to DNA structure alteration or nicking during PT incorporation [5,12]. But unfortunately, for some unexpected reasons (for example, it is difficult to get these proteins with right global folding), the structures of these three proteins have never been reported, which obviously delays research scientists from understanding the pathway of DNA PT modification in bacteria.

In comparison with DndB, DndC and DndD, it seems that the function of DndA is much easier to be predicted. DndA is likely

involved in the first step of DNA phosphorothioation. It works as a cysteine desulfurase and assembles DndC as a 4Fe-4S cluster protein [8]. This prediction was confirmed by the newly determined crystal structure of *Streptomyces lividans* DndA in complex with its covalently bound cofactor PLP [13]. This structure shows a potential interaction site on DndA for the substrate L-cysteine. However, interestingly, in this crystal structure, the catalytic cysteine in DndA locates at a β strand (which is more rigid than loop), distinct from those in other previously reported cysteine desulfurases (where it always resides in a loop). This catalytic cysteine is much far away from the presumable location of the substrate, suggesting that a conformational change of DndA is required during the catalysis process to bring the catalytic cysteine close to the substrate cysteine. However, based on the results of the *in vitro* enzymatic studies, this conformational change is unlikely resulted from random thermal motion, because moving the catalytic cysteine two residues forward or backward in the primary sequence may completely delete the cysteine desulfurase activity of DndA. So, to confirm whether the conformational change is happened or not during catalysis, combined with X-ray and other techniques, further investigations on the NMR chemical shift changes of the residues, dynamics properties, and even local conformation determination in free and bound (with L-cysteine) states within this region of DndA might be helpful.

Lastly, DndE has high sequence identity (46%) and similarity (61%) to phosphoribosylaminoimidazole carboxylase (NCAIR synthetase) from *Anabaena variabilis* [11], suggesting that DndE could be a NCAIR synthase analogue [14]. But DndE may also act as a sulfotransferase during DNA PT modification because a specific PAPS binding site with a sequence AAVGK-TLLIHLHR was to be observed in the C-terminus of DndE from *Streptomyces lividans* [10,15]. Therefore, the exact function of DndE could not be excluded based on the bioinformatics studies. To address this question, the crystal structure of DndE from *E coli* B7A was determined in our group [16]. It demonstrates that DndE is neither a sulfotransferase nor a NCAIR synthase analogue, but a possible nicked dsDNA binding protein with an unrecognized fold. Thus, DNA nicking and nicked DNA binding by DndE was suggested to be essential for DNA PT modification. However, how the nicked dsDNA is generated during DNA PT modification? Does it come

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Received September 24, 2012; Accepted September 25, 2012; Published September 30, 2012

Citation: Lan W, Cao C (2012) Studies on DNA Phosphorothioation Modification: Chances and Challenges. Organic Chem Curr Res 1:e115. doi:10.4172/2161-0401.1000e115

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from the regular DNA digestion by DndD? Do DndE and DndD work together to produce nicked dsDNA? All these questions are worthy of further investigations.

In summary, the PT modified DNA functions as an anti-oxidant in bacteria. The reported structures of DndA and DndE cannot describe the pathway of DNA PT modification observed widely in bacteria, but they present chemists and biologists in this area with much more scientific questions, chances and challenges.

Acknowledgement

This work was supported by National Basic Research Program of China under No. 2009CB918600 and 2011CB966300, by National Science Foundation of China under No. 221272261, 20905074 and 20921091, and by National New Drug Design Program from Ministry of Health of China under No. 2011ZX09506.

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