Department of Chemistry: Part III Project 2017/2018

Prof. S. Balasubramanian

Synthesis of caged nucleosides to study the effect of modified bases on DNA conformation

Group web site http://www-balasubramanian.ch.cam.ac.uk

Contact details: Alex Hofer, ah938@cam.ac.uk

In most organisms, nucleic acids are dynamically methylated at various specific sites. These tightly regulated small modifications of the chemical structure of DNA (as well as RNA) can be recognised by various proteins and are important e.g. for the regulation of gene expression. In higher eukaryotes including mammals, the main site of DNA methylation is the nucleobase cytosine (C), which is converted to 5-methylcytosine (5mC).[1] Demethylation of this modified base occurs via an oxidative pathway, and it was shown in our group that the demethylation intermediates (e.g. 5-hydroxymethylcytosine (5hmC) and 5-formylcytosine (5fC)) can alter the native conformation of DNA and also affect chromatin regulation and transcription.[2,3]

The methylated base N^6-methyladenine (6mA) has long been known to be present in the DNA of bacteria and some lower eukaryotes, but its presence in higher eukaryotes and possible regulatory roles were established only recently.[4–7] Demethylation of this base in RNA has been shown to occur via the oxidative intermediates N^6-hydroxymethyladenine (6hmA) and N^6-formyladenine (6fA), two chemically unstable intermediates that slowly hydrolyse to adenine (A) in aqueous medium, releasing formaldehyde or formic acid, respectively (see Figure 1).[8] Half-life times of 6hmA and 6fA in water under near-physiological conditions have been estimated to several hours. Thus, they are expected to be present *in vivo* long enough to affect the native conformation of DNA and play a regulatory role.

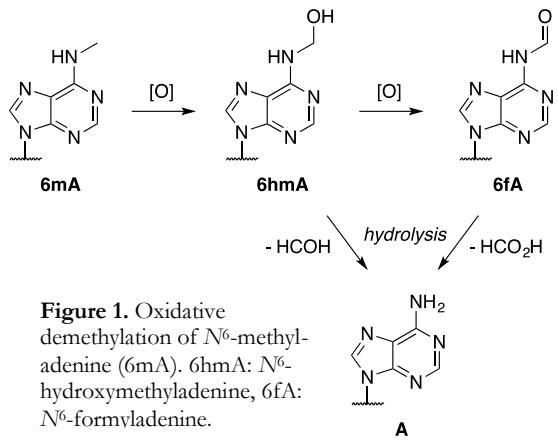

Figure 1. Oxidative demethylation of N^6-methyladenine (6mA). 6hmA: N^6-hydroxymethyladenine, 6fA: N^6-formyladenine.

For this Part III project, we propose to design and synthesise DNA nucleosides with appropriate protecting groups or cages to release the transient intermediates in a temporally controlled fashion (see Figure 2). Such caged nucleosides could then be introduced in a DNA strand by preparing the corresponding phosphoramidites and using them in solid phase oligonucleotide synthesis. This would allow obtaining DNA fragments with the unstable nucleobases 6hmA and 6fA to study potential effects of these transient species on DNA conformation.

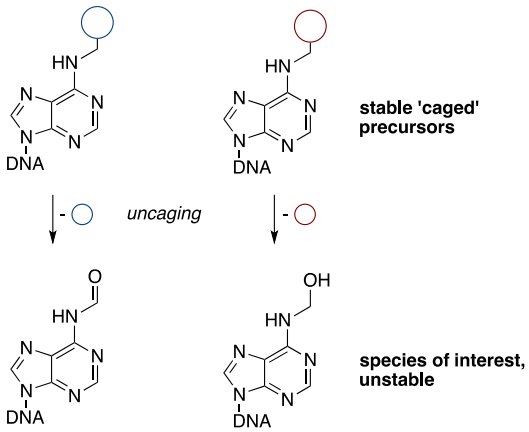

Figure 2. Proposed development of caged nucleosides releasing 6fA and 6hmA in a temporally controlled fashion.

[1] D. Schübeler, *Nature* **2015**, *517*, 321–326.
[2] M. Iurlaro et al., *Genome Biol.* **2013**, *14*, R119.
[3] E.-A. Raiber et al., *Nat. Struct. Mol. Biol.* **2015**, *22*, 44–49.
[4] Y. Fu et al., *Cell* **2015**, *161*, 879–892.
[5] E. L. Greer et al., *Cell* **2015**, *161*, 868–878.
[6] G. Zhang et al., *Cell* **2015**, *161*, 893–906.
[7] G.-Z. Luo, M. A. Blanco, E. L. Greer, C. He, Y. Shi, *Nat. Rev. Mol. Cell Biol.* **2015**, *16*, 705–710.
[8] Y. Fu, et al., *Nat. Commun.* **2013**, *4*, 1798.