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FINAL REPORT: Determination of (5'S)- and (5'R)-5',8-cyclo-2'-deoxyadenosine ratio in DNA oxidative damage

The aim of the visit was to set up a sensitive and accurate analytical protocol for the quantitative determination of (5'*S*)- and (5'*R*)-8-cyclo-2'-deoxyadenosine lesions in single- and double-stranded DNA exposed to hydroxyl radicals generated by γ-irradiation of aqueous solutions and to study the influence of the experimental conditions and microenvironment on the ratio of the (5'*S*)- and (5'*R*)-diastereoisomers.

In order to set up an efficient enzymic digestion of γ-irradiated DNA samples containing the above mentioned lesions we started working on a variety of single stranded oligodeoxynucleotides containing either the (5'R) or the (5'S)-cyclo-2'-deoxyadenosine (cdA) (TAAATTGcdACTGACTGACGC; ATCGTGcdACTGATCT; CGTACTcdATGAGC; TcdAT ; TcdA).

All the digested samples have been analysed by HPLC/MS/MS. Quantitative analyses have been performed using pure samples of (5'*S*)- and (5'*R*)-cdA and TcdA, previously prepared as external standards.

The enzymic digestibility of oligodeoxynucleotides containing (5'*R*)-cdA was found up to 95% using Dase II, Phosphodiesterase II, Nuclease PI and Phosfatase alcaline. On the contrary, some problems were found in the case of oligodeoxynucleotides containing (5'*S*)-cdA. In particular, the release of (5'*S*)-cdA when in 5' position is present a guanosine or a thymidine moiety was not efficient with the protocol above mentioned. By increasing the amount of nuclease PI and the digestion time we were able to optimise the procedure in the case of the 5'-guanosine obtaining almost a quantitative release of (5'*S*)-cdA. In the case of 5'-thymidine the same modified protocol allowed to obtain the dimer

TcdA up to 85% and (5'*S*)-cdA in 15% yield. On this bases, this protocol can be considered accurate enough to proceed with the DNA-irradiated analyses. However, we will make another attempt to improve the digestibility of TcdA using acidic phosfatase in addition to nuclease PI.

By the way, we decided to extend this study to the (5'*S*)- and (5'*R*)-8-cyclo-2'-deoxyguanosine. (5'*S*)-8-cyclo-2'-deoxyguanosine has been previously prepared through a multistep synthesis in my laboratory at CNR in Bologna. Moreover, I prepared a pure sample of (5'*R*)- 8-cyclo-2'-deoxyaguanosine by γ-irradiation of aqueous solution of 2'-deoxyaguanosine under deoxygenated conditions and subsequent preparative HPLC purification during my stay in Grenoble. The appropriate HPLC/MS/MS method has been set up. We have already started with some preliminarily enzimyc digestion of oligodeoxynucleotides containing (5'S)-cdG (ATCGTcdGACTGATCT).

The second part of the project, consisting in the quantitative determination of the 2'-deoxyadenosine- (and 2'-deoxyguanosine-)cyclonucleosides and the (5'*S*)/(5'*R*) diastereoisomeric ratio in DNA exposed to hydroxyl radicals generated by γ-irradiation of aqueous solutions will be carried on in collaboration between Bologna and Grenoble. In particular, the γ-irradiation experiments under different oxygen concentrations will be performed in Bologna whereas the samples will be digested and analysed in Grenoble.

As soon as we have the results on DNA, attempts will be made to look for the radiation-induced formation of (5'*S*)- and (5'*R*)-5',8-cyclo-2'-deoxyadenosine and guanosine lesions within cellular DNA.