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Combination of chromatographic and chemometric methods to study the interactions between DNA strands

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Introduction

DNA strands can form secondary structures of varying complexity. It is well known the double helix structure discovered by Watson and Crick in which two complementary DNA strands interact. However, besides this duplex structure, there are more complex arrangements such as triplexes (interaction of three strands) or quadruplexes (interaction of four strands). In recent years, it has been proposed that these structures could be very important "in vivo" as they could be used in antigenic therapies or, also, could be closely related to the appearance of certain types of aging cancer[1-2].

Thus, we studied the formation of these structures considering two different options: a) the formation of duplex structures from the interaction of two single-stranded DNAs and b) the competition of duplex and quadruplex structures. These structures have different size and weight and, so, it will be possible to separate them in a size exclusion chromatographic analysis.

In some cases, the analysis of the chromatogram at a single wavelength is enough to confirm the existence of a higher order structure. In other cases, it is necessary to acquire the entire spectrum at each point of the chromatogram and to apply chemometric resolution methods in order to detect the formation of the complex structures.

Experimental

The following DNA sequences has been used in this work:

s14A: 5' - CCGCGATATGCCGCC – 3' which adopts a random coil structure
s14B: 5' - GGCGCATATCGCGG – 3' which adopts a random coil structure
hTERTG: 5' - AGGGGAGGGGCTGGGAGGGC – 3' which folds into a intramolecular G-quadruplex structure
hTERTC: 5' - GCCCTCCCCTCCCCTCCCCT – 3' which folds into a random coil structure at neutral pH or a intramolecular i-motif structure at acidic pH

s14A/s14B and hTERTG/hTERTC are complementary sequences and will form the duplex structure if they are in the same solution.

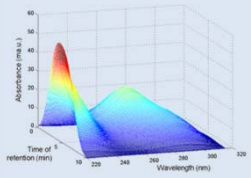
Procedure

The chromatographic system consisted of an Agilent 1100 Series HPLC instrument equipped with an Agilent Chemstation for data acquisition. A Phenomenex BioSep-SEC-S 2000 column (300x7,8 mm, particle size 5 µm and pore size 145 Å) from Phenomenex (Torrance, CA, USA) was used for the chromatographic separation. The mobile phase was a pH 7 buffer (phosphate 0.1 M with 150 mM of potassium or sodium depending on the experiment). Flow rate was 1.3 mL/min, 15 µl of the sample were injected and the temperature was 25 ˚C. Chromatograms were recorded between 220 and 500 nm.

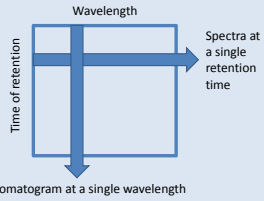
Each experiment consisted in the injection of samples of the pure oligonucleotides (sytems s14A/s14B and hTERTG/hTERTC) and different mixtures of the complementary oligonucleotides covering the ratio of concentrations of between 0 and 3:1.

Data Analysis

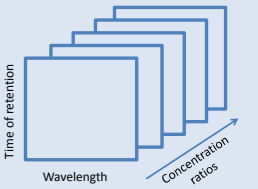
From each injection a 3D surface is obtained



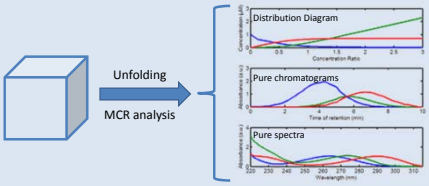
This can be represented as a data matrix



Each experiment creates a data cube



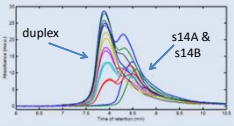
Multivariate Curve Resolution chemometric method is used to recover the distribution diagram of the mole-ratio experiment and the pure chromatograms and spectra of the different species[3-5].



Results

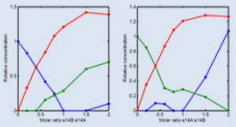
Experiments of duplex formation

Experimental data considering the chromatograms at 260 nm.



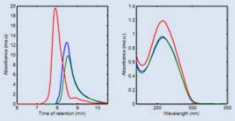
The largest molecule (duplex) elutes before than s14A and s14B single strands (these two elute at the same time of retention)

MCR resolved distribution diagram of the mole-ratio experiment



The formation of the duplex is resolved and reaches its maximum concentration at the ratio 1:1.

MCR resolved pure chromatograms and spectra



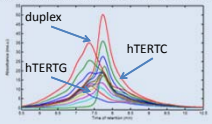
Single strands show similar chromatograms and spectra whereas the duplex species has a different spectrum and chromatogram .

Legend: s14A, s14B, duplex

Experiments of duplex:quadruplex competition

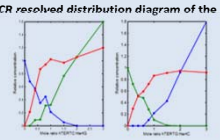
Competition in potassium medium

Experimental data considering the chromatograms at 260 nm.



hTERTG shows a broad peak between 6 and 8.5 minutes (multiple structures are present, such as different G-quadruplexes and the single strand). hTERTC shows a sharp peak at 8 minutes (single strand).

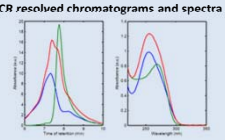
MCR resolved distribution diagram of the mole-ratio experiment



Distribution diagram shows clearly the apparition of the duplex structure formed by the hTERTG and hTERTC sequences.

Resolved chromatograms clearly show the size order of the molecules: G-quadruplex (hTERTG)> duplex > single strand (hTERTC).

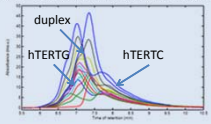
MCR resolved chromatograms and spectra



hTERTG shows the contribution of more than one structures. The peak corresponding to the duplex appears between the hTERTG and hTERTC (despite it is not totally well resolved).

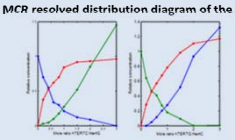
Competition in sodium medium

Experimental data considering the chromatograms at 260 nm.



hTERTG shows a peak with two components (G-quadruplex and single strand). hTERTC shows a sharp peak at 8 minutes (random coil single strand).

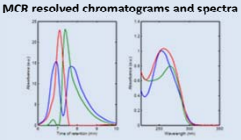
MCR resolved distribution diagram of the mole-ratio experiment



Distribution diagrams are similar to those obtained in the potassium medium with the formation of the duplex structure.

Resolved chromatogram of hTERTG shows clearly the two contributions. In this case, both duplex and hTERTC peaks are correctly resolved.

MCR resolved chromatograms and spectra



This better resolution compared with the potassium case can be explained by the sharper peaks of hTERTG.

Legend: hTERTG, hTERTC, duplex

Conclusions

This work has demonstrated the usefulness of the combination of chromatographic and chemometric techniques to study the interactions between complementary DNA strands although the chromatographic peaks and the spectra are overlapped.

In all the cases, the soft-modelling method Multivariate Curve Resolution by Alternating Least Squares has allowed the resolution of the system identifying three different species. In the case of the study of the quadruplex forming sequence (hTERTG), a different behaviour in the two cationic medium (sodium and potassium) has been observed so that in potassium medium the quadruplex structure is favoured.

However, there is still more work to do:

- Study the formation of triplex structures which are more difficult to detect spectroscopically from the mixture of duplex and single strand structures.
- Fusion of the data obtained by liquid chromatography and diode array detection with other techniques such as mass spectrometry in order to confirm the results.

References

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Acknowledgements

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