Study of β-NMR for Liquid Biological Samples

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Abstract

β-NMR is an exotic form of NMR spectroscopy that allows for the characterization of matter based on the anisotropic β-decay of radioactive probe nuclei. This has been shown to be an effective spectroscopic technique for many different compounds, but its use for liquid biological samples is relatively unexplored. The work at the VITO line of ISOLDE seeks to employ this technique to study such samples. Currently, preparations are being made for an experiment to characterize DNA G-quadruplexes and their interactions with stabilizing cations. More specifically, the work in which I engaged as a summer student focused on the experiment's liquid handling system and the stability of the relevant biological samples under vacuum.

Introduction

The biological molecule to be studied using β-NMR at VITO is the DNA G-quadruplex. G-quadruplexes are secondary structures observed in guanine rich areas of nucleic acids. Guanines hydrogen bond with each other as seen in Figure 1to form helices of four nitrogenous bases per turn, a single iteration of which is seen in Figure 2. These iterations layer on top of each other, stabilized by an interior cation. G-quadruplexes are often found in regulatory and telomeric regions of oncogenes, making them a particular subject of interest. It should be noted, however, that the interactions of these quadruplexes with the interior cations are not clearly seen using conventional NMR, so other methods of imaging must be employed. β-NMR immediately presents itself as a plausible method, largely due to the nature of the interior cations of the G-quadruplexes. In this case, the experiment will be using various isotopes of Na as probe nuclei to characterize the cation-DNA interaction. Na is a convenient choice because it has multiple unstable isotopes with experimentally viable half lives as well as non-zero spins, both desirable characteristics for β-NMR.

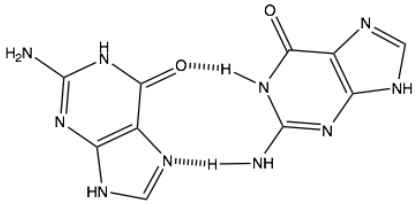

Figure 1. Guanines hydrogen bonding.

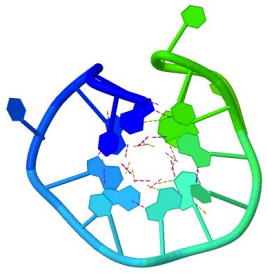

Figure 2. Single iteration of a DNA G-quadruplex. Imaged using data from conventional NMR (1).

NMR Spectroscopy

Conventional NMR is an incredibly powerful technique, but as seen in the case of G-quadruplexes, has limitations that render certain nuclei virtually invisible. It is most readily applied to nuclei of spin ½, and while it can be used for those with greater spin, the quadrupole interactions of these nuclei significantly reduce the signal to noise ratio. This is remedied in

β-NMR by the fact that radioactive nuclei are laser-polarized and then implanted into the sample, as opposed to being polarized directly in the sample using a magnetic field. This allows for a much higher degree of polarization and therefore a clearer signal by several orders of magnitude. The detection method of β-NMR also differs from that of conventional NMR spectroscopy, relying on the anisotropy of β-decay of the probe nuclei, as opposed to the emission of a specific frequency of electromagnetic energy with the relaxation of polarized nuclei. It is important to note, however, that the improved signal resolution deriving from the choice of β-NMR over conventional NMR introduces some technical difficulties, having hitherto prevented its wide application to biological samples. Because most such samples exist in aqueous form, a mechanism for keeping these liquid samples stable under vacuum must be devised. Typical methods for doing such, ie using a jet of fluid as opposed to a drop, are not sufficient because the sample must remain stationary for at least the period of a few half-lives to allow for appropriate β detection.

Liquid Handling Tests

The original configuration of the liquid handling system involved the ejection of the sample through a capillary using a precision titration device, allowing it to rest as a drop at the tip of the capillary in the vacuum chamber. Tests of this system, however, revealed difficulties in maintaining control while under vacuum. Currently, manipulations of the system seek not only to maintain a stable drop, but also to hold the sample in such a configuration as to maximize the exposure of beam to biomolecule. The first attempted solution involves the installation of a capillary support system on which the drop can rest, as shown in Figure 3. The hook shape allows the drop to be drawn out as a thin film, exposing more of the DNA to probe nuclei than in the case of a spherical drop. Testing of this system has shown that water samples hold stable down to ~10 mbar, but testing at lower pressures still needs to be done. A drawback of this setup is that the method of creating the capillary support, heating the glass and allowing it to deform under gravity, is rather imprecise. This makes it difficult to maximize the parameters of the support according to the needs of the experiment. At present, the most successful configuration that has been made is the hook shown in Figure 3. The beam has a diameter of 3mm, which is approximated by this hook, but as can be seen it is not exact.

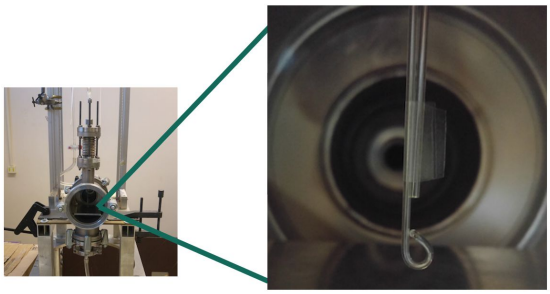

Figure 3. Capillary support system.

Another setup that has been designed includes the introduction of a substrate on which the liquid drop can rest, as seen in Figure 4. This has not yet been tested, but the current idea is to use a glass filter as such a substrate. This should in effect work as a molecular sieve, dehydrating the sample slightly and allowing for the biomolecules to rest stably atop the substrate. This has

the advantage of providing a rest for the sample so that gravitational troubles become negligible, as well as providing a hydrophilic surface to which the sample will hopefully cling when put under vacuum.

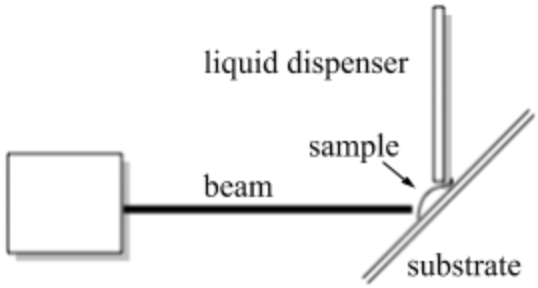

Figure 4. Substrate-droplet configuration. Side view.

It should also be noted that while these configurations are believed to work with aqueous solution, the properties of the sample will vary considerably with the actual contents of said sample. For this reason, testing with various ionic liquids is being discussed. Currently, [emim][Ac] and [emim][PF_6] have been chosen for their properties at room temperature as well as their demonstrated chemical shift. There are certain limitations, however, to the extent of manipulations that can be made to the sample itself. This is because it is biological in nature, and while certain fluid combinations may provide for a more stable setup/better imaging, it is possible that these lose their biological relevance on account of pH, temperature, etc.

Conclusions

β-NMR is a very powerful technique, but poses significant technical challenges for its application to biological samples. In the case of the G-quadruplexes that will be studied at the VITO beamline, the capillary support system appears to be an effective addition to the liquid handling system for the purposes of overcoming some of these challenges. It is, however, imprecise and does not eliminate all control issues. More testing, particularly as concerns the substrates, must be done in order to make conclusions about the most effective option. It is also worth considering a setup that employs both methods in tandem.

References

1. Image from the RCSB PDB (www.rcsb.org) of PDB ID 2MS9 (W.J. Chung, B. Heddi, E. Schmitt, K.W. Lim, Y. Mechulam, A.T. Phan) (2015) Structure of a left-handed DNA G-quadruplex *Proc.Natl.Acad.Sci.USA*.

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