

Thrombus detection using Fast Field-Cycling NMR

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Introduction

Aggregated proteins are central to several diseases and early detection is of great interest for diagnosis. This research investigates the possibility of detecting protein aggregation by using fast field-cycling (FFC) nuclear magnetic resonance relaxometry. FFC is a technique that consists of changing the strength of the main magnetic field during the pulse sequence. It offers many possibilities since it allows using low evolution fields to investigate the dispersion of the relaxation rate, and switching back to a higher field during the detection phase to provide a high signal-to-noise ratio in the data. This technique is applied to the detection of the ^{14}N quadrupolar relaxation process [1], which can be seen in various biological samples as characteristic peaks in the R_1 spectrum of water ^1H , called quadrupolar peaks such as the ones represented in Figure 2. The present work aims to check the linear relationship suggested by the theory [2,6] between the amplitude of the ^{14}N quadrupolar signal generated from clotted human fibrin, the main constituent of thrombus, with its concentration using FFC NMR and investigating whether other factors affect amplitude. The wide literature available about fibrin provides much detail about the model system of the formation of fibrin clots in blood clots and wound healing [3]. As a protein, fibrin is rich in ^{14}N and its mobility is reduced due to the web-like structure of a clot so it is a potential source of ^{14}N quadrupole dipoles in a ^1H T_1 dispersion plot [4]. Clots are stabilized and strengthened by plasma protein Factor XIII (FXIII) which covalently cross-links flanking fibrin units [5].

Methods

Samples of clotted human fibrinogen were prepared through cleavage by an enzyme, thrombin, and analysed using an FFC NMR relaxometer (SMARtracer, Stelar S.r.l., Italy). This provided a measure of the T_1 dispersion curve between 1.5 and 3.5 MHz, which included the region of the two main quadrupole dipoles of ^{14}N (at 49 mT and 65 mT – i.e. 2.1 MHz and 2.8 MHz), using an inversion-recovery pulse sequence. Fibrinogen samples were used as a baseline. The determination of the relationship between fibrin concentration and dip amplitude was investigated by preparing samples with differing concentrations of fibrinogen (between $0.4 \text{ mg}\cdot\text{mL}^{-1}$ and $20 \text{ mg}\cdot\text{mL}^{-1}$) and monitoring the corresponding quadrupole dip amplitude. The addition of FXIII ($1 \text{ U}\cdot\text{mL}^{-1}$) and FXIII inhibitor (1 mM) to fibrinogen ($10 \text{ mg}\cdot\text{mL}^{-1}$) and the effect on the peak amplitude was observed.

Results

The samples containing clotted fibrin exhibited peaks in the region of 1.5 to 3 MHz, corresponding to the ^{14}N quadrupole peaks [2] and the linearity between peak amplitude and fibrin concentration is shown in Figure 1. Non-clotted samples did not present such features, which indicate the specificity of this measure for immobilised proteins. Figure 2 shows almost superimposed R_1 peaks indicating no significant difference in the quadrupolar amplitude when FXIII and inhibitor were added to fibrin.

Conclusions

The presence of quadrupolar peaks in dispersion plots of human fibrin has been demonstrated and its linearity has been established over a range of concentrations that covers physiological concentrations (Fig 1). This agrees with the theory [2,6]. The presence or absence of fibrin cross linking does not lead to a change in dip amplitude (Fig 2), which suggests that additional rigidity/ tightness of the clot has no effect on the amplitude of the quadrupolar peaks. This could potentially lead to a novel method using FFC MRI to estimate the concentration of in vivo fibrin, which has clinical relevance for thrombus detection and treatment.

Acknowledgements

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References

- [1] Kimmich R., *Bull Magn. Res.* 1, 1980, 195-218
- [2] Koenig S., *Biophys J.* 53, 1988, 91-96
- [3] Blombäck, B., et al., *Thrombus Research* 1994, 75, 521-538
- [4] Winter F. and Kimmich R., *Biochim. Biophys. Acta* **719**, 1982, 292-298
- [5] McDonagh J., *Hemostasis and Thrombosis* 3rd edn, 1994: 301–11.
- [6] Sunde EP and Halle B, *J. Mag. Res.* **203**(2), 2010, 257-73

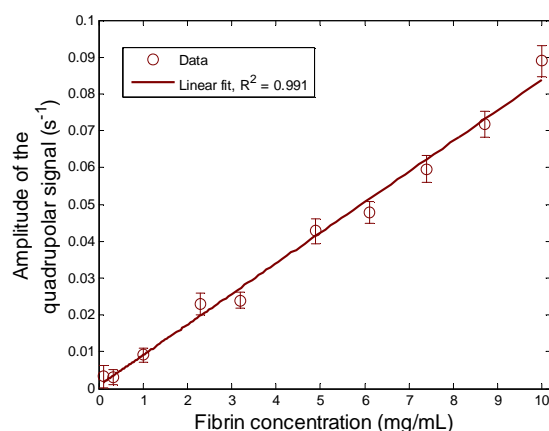


Fig 1: Amplitude of the quadrupolar signal measured for different concentrations of clotted fibrin compared to theoretical fit

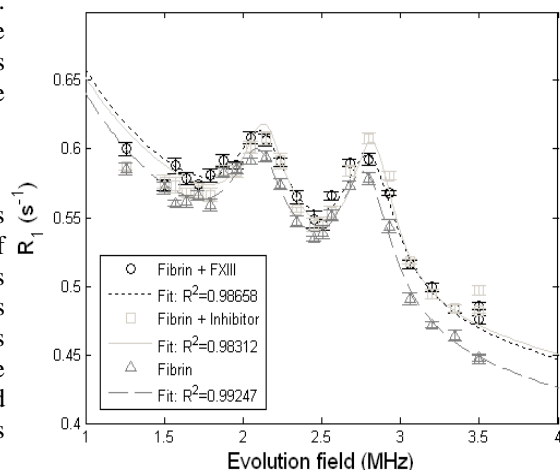


Fig 2: R_1 measured in the quadrupolar dip region of $10 \text{ mg}\cdot\text{mL}^{-1}$ fibrin with added FXIII and FXIII inhibitor