A novel relaxometric method for the measurement of biochemical reactions based on functionalized magnetic nanoparticles

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INTRODUCTION

The antibody/antigen reaction is extensively exploited in various immunoassay techniques with application in biomedical research and clinical diagnosis. The reaction is commonly detected due to a label that is conjugated to the antibody or its antigen. Traditionally, the labels are either radioisotopes or fluorescent, chemiluminiscent and enzyme labels. During the last two decades, enzyme immunoassays with a colorimetric end point have become very popular because they are relatively cheap and easy to handle without the hazards associated with radioisotopes. Nevertheless, these tests are often time-consuming and tend to be less sensitive than those based on radioisotope labels. There is, therefore, a considerable interest in the development of alternative simple and rapid analytical techniques that can be easily implemented into biomedical laboratories. In a new generation of immunoassays, magnetic micro- and nanoparticles are being used as a label and as the solid phase material where an immune reaction can take place. The molecular interaction is directly detected with a force-amplified biosensor, a Maxwell bridge, a super-conducting-quantum-interference-device (SQUID), an electronic detection system or by NMR (nuclear magnetic resonance) relaxometry [1, 2]. Based on this scientific and technical background, the purpose of the present work was to develop new magnetic nanosensors and adequate techniques to measure the molecular interactions, essentially relied on NMR relaxometry.

MATERIAL AND METHODS

Ultra-small particles of iron oxide (USPIO) conjugated to biomolecular probes [streptavidin (USPIO-g-Strp), biotin (USPIO-g-Bt)] were prepared and used to estimate biochemical parameters (i.e. concentration of molecules in biological fluids, expression of cell receptors, evaluation of affinity constants) by the intermediary of T_2 relaxation times measured at 60 MHz on a Bruker Minispec mq60 equipment (Bruker, Karlsruhe, Germany). The biomolecules were grafted to magnetic nanoparticles by reaction with the dextran coating of USPIO previously treated with epichlorhydrin.

RESULTS: Measurement of IgG concentration

In the first experimental protocol, we have developed a technique able to measure the concentration of molecules in biological fluids. For this purpose, human IgG were biotinylated (IgG-Bt) and various dilutions (ranging between 1837 nM and 10 nM) of the stock solution were incubated (3 min, room temperature) with 10 µM of USPIO-g-Strp and 40 µM of USPIO-g-Bt. The macromolecular assembly between these molecules produced a significant increase of the relaxation rate ($R_2 = 1/T_2 = 9.3\ s^{-1}$ at the highest IgG-Bt concentration) as compared to that of USPIO-g-Strp and USPIO-g-Bt ($R_2 = 4.7\ s^{-1}$) in the absence of IgG-Bt. This R_2 enhancement was significantly correlated with IgG-Bt concentration ($r^2 = 0.986$) and the technique was highly sensitive to concentrations as low as 10 nM (Figure 1A).

For a quantitative application of the protocol, we have assessed various methods of R_2^{Norm} expression as a function of IgG-Bt concentration. The one that allowed an accurate theoretical estimation of this biochemical parameter on the basis of R_2 measured at 60 MHz is represented in Figure 1B.

Figure 1 (A, B). Correlation between the R_2 values measured at 60 MHz and the concentrations of IgG-Bt

$R_2^{Norm} = (R_2^{sample} - R_2^{H2O}) - (R_2^{CA} - R_2^{H2O})$

CA = contrast agent

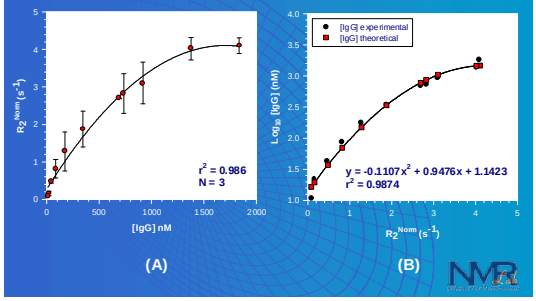
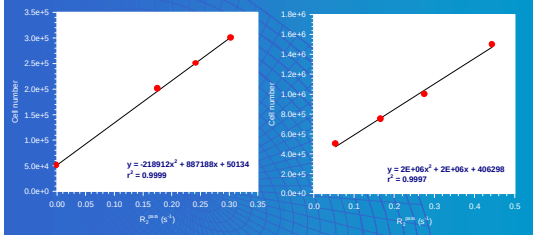


Figure 2 (A, B). Correlation between the R_2 values measured at 60 MHz and cell number



(A) = 2x10^5 cells labeled with 10 µL anti-CD3 / 100 µL

(B) = 2x10^6 cells labeled with 10 µL anti-CD3 / 100 µL

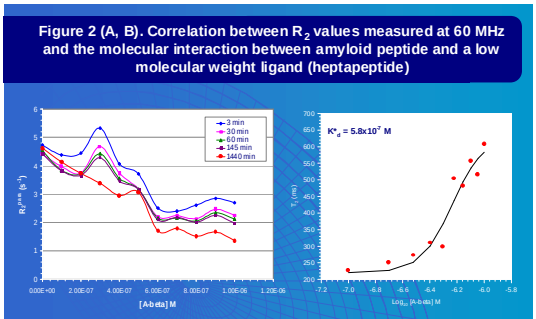
RESULTS: Measurement of cell receptors

To measure the expression of cell receptors by NMR relaxometry, Jurkat T cells were first incubated with mouse anti-human CD3 antibody biotin-conjugated (Chemicon Europe, Heule, Belgium), which recognizes the T cell receptor. The non-bound antibody was removed by centrifugation, and various number of cells ($1.5x10^6$ – $5x10^4$) were then incubated (60 min, room temperature) with 10 µM of USPIO-g-Strp and 40 µM of USPIO-g-Bt. The R_2 was measured after rinsing the non-bound iron nanoparticles and their removal by centrifugation. A good correlation ($r^2 > 0.9$) was found between R_2 and cell number, which means that cell receptors can be accurately estimated by relaxometry. The sensitivity of the method was enhanced when the number of cells labeled with anti-CD3 was diminished (Figure 2 A, B).

RESULTS: Measurement of affinity constants

The measurement of affinity constants was attempted with a heptapeptide (P22) specific for amyloid peptide (A-beta, the major component of the senile plaques in Alzheimer disease), which was selected by phage display [3]. The affinity of biotinylated P22 for its target was determined by relaxometry after labeling with 10 µM USPIO-g-Strp and 30 µM USPIO-g-Bt. The molecular assembly between these molecules indicated a more than three-fold prolongation of T_2 (diminished R_2) in the presence of 1 µM A-beta. The results obtained after 24 h of incubation were used to evaluate the apparent dissociation constant (K^*_d) for this interaction (Figure 3 A, B).

Figure 2 (A, B). Correlation between R_2 values measured at 60 MHz and the molecular interaction between amyloid peptide and a low molecular weight ligand (heptapeptide)



(A) = The time evolution of R_2^{sus} as a function of amyloid peptide concentration

(B) = The estimation of K*d for the molecular interaction between A-beta and the peptide ligand

CONCLUSIONS

To conclude, our results point out the potential of magnetic-based immunoassays as a highly attractive tool for biomedical applications due to a wide range of advantages, such as the minimal sample handling, avoiding of washing steps, direct measurement, cost-effective, and high sensitivity.

REFERENCES

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ACKNOWLEDGEMENTS

This work was financially supported by the ARC program of the French Community of Belgium (research contract no. 00-05/258), by the DGTRE (Region of Wallonia, NOMADE project), and by the project of co-operation CNRS/CGRI-FNRS (research contract no. 12/02/2003-022-5, ref. PV/EJ/FR/1522/ng dossier 03/007).