Streptavidin-based magnetic nanosensors as an useful tool in clinical diagnosis

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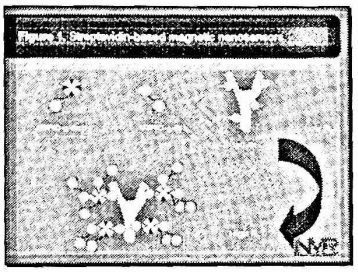
INTRODUCTION

Various metal or semiconductor nanoparticles are currently used as chemical sensors in biomedical applications after coupling to affinity ligands. Molecular interactions result in larger nanoassemblies, which can be sensed in a real-time manner by nuclear magnetic resonance (NMR) if the interacting molecules are magnetically labeled with ultra small particles of iron oxide (USPIO). Various types of targeted iron oxides have lately been developed and used as magnetic labels due to their high transverse relaxivities (r_2), which facilitate the detection of cellular receptors at concentrations as low as 10^{-8} M [1]. The high affinity recognition of biotin by streptavidin has made this protein one of the most useful tools in biotechnology [2]. In the present work, streptavidin (Strp) and biotin (Bt) grafted USPIO particles (USPIO-g-Strp and USPIO-g-Bt) were used to detect and quantify human IgG by NMR relaxometry and imaging (MRI) (Figure 1).



METHODS

The dextran-coating of USPIO particles was crosslinked with epichlorhydrin and aminated to allow for the grafting of Strp and Bt. The particle size of USPIO-g-Strp and USPIO-g-Bt is about 30 nm. Their r_2 at 60 MHz and 37°C is 91.2 s^{-1} mM^{-1} and 77.9 s^{-1} mM^{-1}, respectively. IgG were biotinylated (IgG-Bt) and solubilised in a physiologic solution at a concentration of 600 nM. Various dilutions (ranging between 642 nM and 10 nM) of the stock solution were incubated at 37°C with different concentrations of either USPIO-g-Strp (50, 25, 10 µM) alone or USPIO-g-Strp and USPIO-g-Bt (100, 75, 40 µM). T_2 relaxation times were measured at 60 MHz on a Bruker Minispec mq60 (Bruker, Karlsruhe, Germany) before and after the addition of USPIO solutions. The time-dependence of molecular interaction was followed up for 30 min. For MRI, various dilutions of IgG-Bt (642 nM - 5 nM) were immobilized on protein A-coated ELISA plates and incubated at 37°C with 1 mM USPIO-g-Strp (2h) and 1 mM USPIO-g-Bt (30 min); after each incubation, the unbound ligands were rinsed 3 times with 200 µl PBS. The bound ligands were resuspended with 100 µl of 0.2 M glycine, pH 2.2, neutralized with 15 µl of 1M Tris-HCl, pH 9.1, and analyzed by MRI (Bruker AVANCE-200, 4.7 T, TR/TE = 3000/20 ms, 40 echoes); the T_2 values were measured on images. The Fe concentration was determined in each sample with a calibration curve obtained by MRI by measuring the transverse relaxation rates (R_2) as a function of iron concentration (range of concentration between 0.5 and 0.025 mM). The affinity constants of this interaction were estimated.

RESULTS

The interaction between USPIO-g-Strp ([Fe] = 50 µM) and USPIO-g-Bt ([Fe] = 100 µM) enhanced the R_2 measured by relaxometry up to 15.2 s^{-1} at T_0 as compared to 4.3 s^{-1} and 9.4 s^{-1}, respectively, for individual components at the same concentration. The R_2 of this nanoassembly diminished to 12 s^{-1} 30 min after incubation at 37°C (Figure 2).

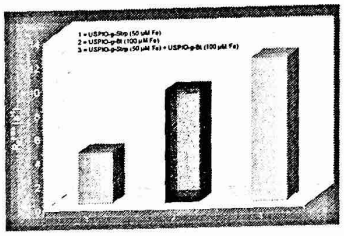


Figure 2. The R_2 (60 MHz, 37°C, 30 min after incubation) of the solution containing 50 µM USPIO-g-Strp and 100 µM USPIO-g-Bt as compared to the R_2 of individual components at the same concentration.

The addition of 50 µM USPIO-g-Strp to IgG-Bt raised R_2 in a concentration-dependent manner, i.e. 5.5 s^{-1} for 642 nM IgG-Bt, 5.1 s^{-1} for 321 nM IgG-Bt (Figure 3).

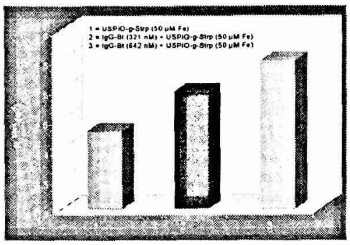


Figure 3. The R_2 (60 MHz, 37°C, 30 min after incubation, TE = 0.6 ms, TR = 15 s, 500 echoes) of the macromolecular assembly between 50 µM USPIO-g-Strp and different concentrations (i.e. 321 nM, 642 nM) of IgG-Bt as compared to the R_2 of 50 µM USPIO-g-Strp alone.

The macromolecular assembly between IgG-Bt, USPIO-g-Strp, and USPIO-g-Bt induced a significant increase of R_2, which attained a value of 16.6 s^{-1} for 642 nM IgG-Bt, and 13.6 s^{-1} for 321 nM IgG-Bt, respectively (Figure 4).

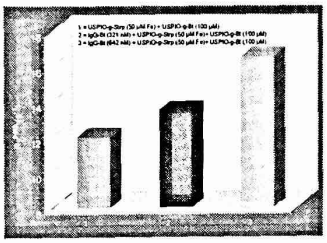


Figure 4. The R_2 (60 MHz, 37°C, 30 min after incubation, TE = 0.2 ms, TR = 6.5 s, 500 echoes) of the macromolecular assembly between different concentrations (i.e. 321 nM, 642 nM) of IgG-Bt, 50 µM USPIO-g-Strp and 100 µM USPIO-g-Bt. The results are compared to the R_2 of the solution containing 50 µM USPIO-g-Strp and 100 µM USPIO-g-Bt.

The bi-exponential fitting of the relaxation curves revealed the existence of two relaxing components (A_1 %, A_2 %). A_1 % (corresponding to the higher R_2, R_{21}) constantly increased during the incubation period (30 min) and it was associated with a slight decrease of R_{21} (Figure 5 a, b, c).

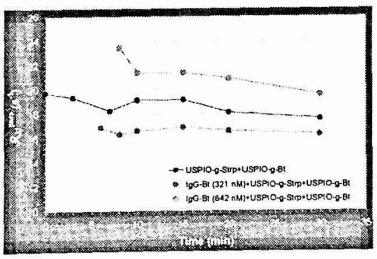


Figure 5 a

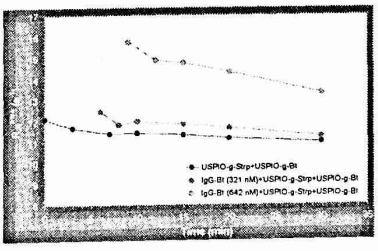


Figure 5 b

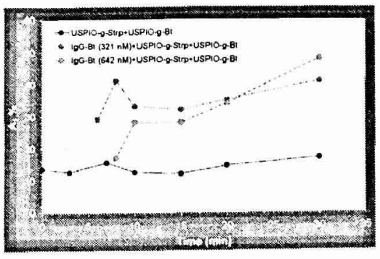


Figure 5 c

Figure 5 a, b, c. The evolution in time of R_{21} (Fig 4a) R_{22} (Fig 4b) and of A_1 % (Fig 4c) (60 MHz, 37°C, TE = 0.2 ms, TR = 6.5 s, 500 echoes) of the macromolecular assembly between different concentrations (i.e. 321 nM, 642 nM) of IgG-Bt, 50 µM USPIO-g-Strp and 100 µM USPIO-g-Bt. The results are compared to the R_2 of the solution containing 50 µM USPIO-g-Strp and 100 µM USPIO-g-Bt.

The calibration curve obtained by incubating various concentrations (693.5 nM – 43 nM) of IgG-Bt with 10 µM USPIO-g-Strp and 40 µM USPIO-g-Bt, evidenced that the magnetic nanosensors are highly sensitive to IgG-Bt concentrations as low as 43 nM (Figure 6).

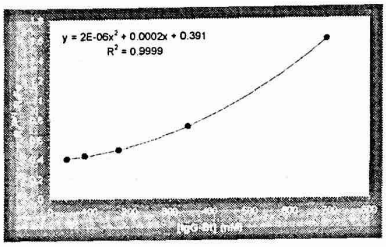
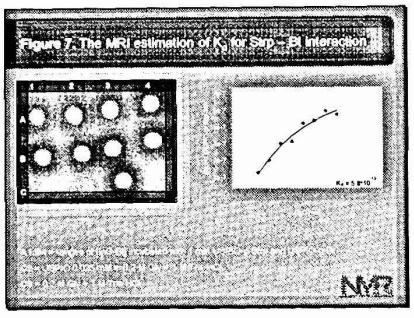


Figure 6. The calibration curve obtained by incubating various concentrations of IgG-Bt with 10 µM USPIO-g-Strp and 40 µM USPIO-g-Bt (60 MHz, 37°C, TE = 0.4 ms, TR = 10 s, 500 echoes).

The K_d estimated by MRI for Strp – Bt interaction was $5.8*10^{-13}$ M, which is quite close to the one mentioned in literature [3], i.e. 10^{-15} M (Figure 7).



CONCLUSIONS

The highly sensitive streptavidin-based magnetic nanosensors described in our work could find various applications in biomedical research and clinical diagnostics.

ACKNOWLEDGMENTS

This work was financially supported by the ARC program of the French Community of Belgium (research contract no. 95/00-194 and 00-05/258).

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