

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

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The Abstract

Introduction: Various types of targeted iron oxides have lately been developed and used as magnetic labels due to their high r_2 facilitating the detection of cellular receptors at concentrations as low as 10^{-8} M [1]. The high affinity of biotin for 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).

Subjects and Methods: Strp and Bt were grafted on USPIO by epichlorhydrin reaction. Various dilutions (642 nM - 10 nM) of biotinylated IgG (IgG-Bt) were incubated with different concentrations of either USPIO-g-Strp alone or USPIO-g-Strp and USPIO-g-Bt. T_2 values were measured (60 MHz, Bruker Minispec) before and after the addition of USPIO solutions. For MRI, various dilutions of IgG-Bt were immobilized on protein A-coated ELISA plates and incubated with 1 mM USPIO-g-Strp and 4 mM USPIO-g-Bt. After rinsing, the bound ligands were resuspended with 0.2 M glycine and analyzed by MRI (Bruker AVANCE-200, 4.7 T); the T_2 values were measured on images. Iron concentration was determined with a calibration curve obtained by MRI. The affinity constants of this interaction were estimated.

Results: The addition of 50 μ M 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, as compared to 4.3 s^{-1} for 50 μ M USPIO-g-Strp alone. The macromolecular assembly between IgG-Bt, USPIO-g-Strp (50mM), and USPIO-g-Bt (100mM) induced a significant increase of R_2 , which reached a value of 16.6 s^{-1} for 642 nM IgG-Bt, and 13.6 s^{-1} for 321 nM IgG-Bt, respectively; the R_2 of the assembly between USPIO-g-Strp and USPIO-g-Bt is of 12 s^{-1} . The results have shown that the magnetic nanosensors are highly sensitive to IgG-Bt concentrations as low as 38 nM. The K_d estimated by MRI (Figure 1) for Strp-Bt interaction was $5.8 \cdot 10^{-14}$ M, which is quite close to the one mentioned in literature [3], i.e. 10^{-15} M.

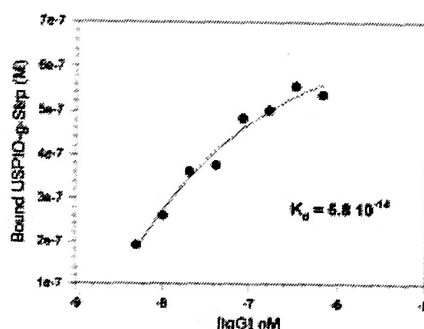


Figure 1. MRI estimation of K_d for Strp-Bt interaction

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

References:

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3. Chalet L, Wolf EJ, [1964] Arch Biochem Biophys, 108, 1-5.

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