

Switching assay as a novel approach for specific antigen-antibody interaction analysis using magnetic nanoparticles

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Abstract. Switching assay was applied for the detection of antigen-antibody interaction between 70-kDa heat shock protein (Hsp70) and anti-Hsp70 monoclonal antibodies in water solutions using conjugates with magnetic iron oxide nanoparticles (MNPs). Hsp70 is a ubiquitous intracellular protein that plays a crucial role in cancerogenesis and many other pathologies. Detection of the Hsp70 level in the biological fluids might have a prognostic and diagnostic value in clinic. The developed switch assay for the detection of Hsp70 demonstrated high sensitivity for antigen-antibody interaction analysis thus proving its potential for further preclinical and clinical studies.

1. Introduction

Switching assay [1] is an accurate and robust technique for detection of water soluble biomarkers in various biological fluids (e.g., blood, urine, etc). Due to magnetic field local inhomogeneities induced by MNPs in water suspensions the proton's transverse relaxation time (T_2) is significantly decreased. The interaction between magnetic conjugates and molecules of the biomarker dramatically affects the relaxation behavior of water protons, thus giving the possibility to detect the biomarker in the solution and measure its concentration. In the presented study the feasibility of the Switching assay for the detection of antigen-antibody interaction between Hsp70 and anti-Hsp70 monoclonal antibody in water solutions was assessed. Molecular chaperone Hsp70 is involved in pathogenesis of many diseases (e.g., cancer, stroke) and its detection represents a high diagnostic and prognostic potential for management of the patients.



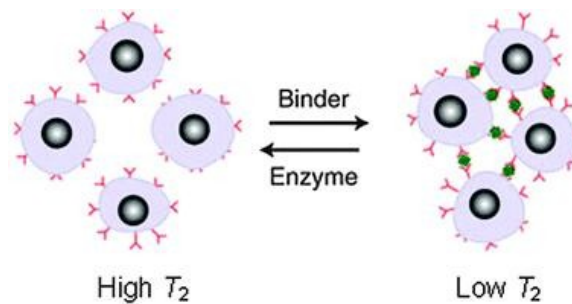


Figure 1. Scheme of magnetic switching assay [2].

2. Materials and methods

Iron oxide MNPs were prepared by co-precipitation of water dissolved Fe(II) and Fe(III) salts and covered with dextran (10kD, Sigma-Aldrich, USA)[3]. Magnetic conjugates of dextran coated MNPs with proteins (Hsp70 or anti-Hsp70 monoclonal antibody) were synthesized by coupling of COO-protein groups to carbodiimide activated surface dextran. The prepared non-conjugated MNPs and protein-decorated nanoparticles were assessed with a high sensitive method of longitudinal nonlinear response of magnetic nanoparticles to a weak *ac* magnetic field with measurements of second harmonic of magnetization (NLR- M_2). The obtained results demonstrated the superparamagnetic properties of the MNPs. The size distribution of the MNPs in suspension was measured by dynamic light scattering (DLS). The distribution for both MNPs coated with dextran and MNPs conjugated with antibodies was bimodal with average hydrodynamic radii of modes equal to 34 nm and 159 nm and 34 nm and 240 nm respectively (Figure 2). Recombinant human Hsp70 was prepared from *E. coli* transformed with a pMSHsp70 plasmid. Anti-Hsp70 monoclonal antibody was produced by hybridoma Mus musculus 2E4 [4]. The prepared suspensions of magnetic conjugates were mixed with Hsp70 (or anti-Hsp70 antibody) and the dynamics of the immune reaction was studied by measuring T_2 time of relaxation using CXP-300 (Bruker) 7.1 T for 90 min. For estimation of proton magnetic relaxation times the inversion recovery and Carr-Purcell-Meiboom-Gill impulse sequences were employed. The magnetic relaxation rate R_2^* was measured from half width of resonance line.

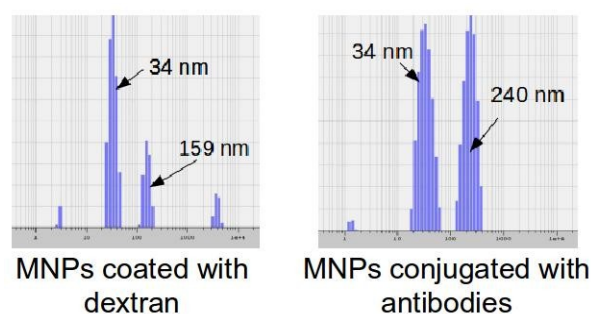


Figure 2. Size distribution of the synthesized MNPs.

3. Results and discussion

In experiment the suspension of MNPs was located in highly homogeneous field B_0 of superconducting magnet of NMR spectrometer. In dipole approximation the MNPs induce additional inhomogeneous field which z component is described by relation

$$B = \frac{\mu M}{3} \left(\frac{R}{r} \right)^3 (3 \cos^2 \theta - 1) \quad (1)$$

where B – magnetic induction, $\mu = 4\pi \cdot 10^{-7}$ H/m, M – magnetization, r – distance from center to reference point, θ – the angle between r vector and direction of constant magnetic field of spectrometer [5],[6]. The water protons in vicinity of MNPs experience the dephasing of spin precession in magnetic field B_0 . The spin-lattice and spin-spin relaxation times T_1 , T_2 and their inverse values R_1 , R_2 are related with static and fast diffusional mechanisms of outer-sphere relaxation theory which leads to formula:

$$R_1 = \frac{1}{T_1} = \frac{32\pi}{135} \gamma_I^2 \frac{n}{RD} \left[3J^A(\sqrt{2\omega_I\tau_D}) \right] \langle \mu_z \rangle^2$$

$$R_2 = \frac{1}{T_2} = \frac{32\pi}{135} \gamma_I^2 \frac{n}{RD} \left[\frac{3}{2} J^A(\sqrt{2\omega_I\tau_D}) + 2J^A(0) \right] \langle \mu_z \rangle^2 \quad (2)$$

where γ_I is the gyromagnetic ratio of the proton, D is the self-diffusion constant of the water, n is the number of MNPs per unit volume, μ is the magnetic moment, R is the effective radius associated with a magnetic nanoparticle, $\tau_D = R^2/D$, and $J^A(z)$ is the spectral density of the correlation function related to molecular diffusion in the inhomogeneous local magnetic field produced by the magnetic particles. The increased relaxivity r_2 of water protons arises from the time fluctuation of the dipolar interaction between the magnetic moment of superparamagnetic domain and proton magnetic moment of the H_2O . The fluctuating local magnetic field in the vicinity of a magnetic core is governed by outer sphere relaxation mechanism of nonhomogeneous broadening in magnetic suspensions. The proton relaxation times T_1 , T_2 , T_2^* of water protons were found to depend on the concentration MNPs in suspension. The magnetic relaxation rates R_1 , R_2 , R_2^* as inverse time relaxation values have shown a linear dependence on total Fe content. The experimental results for R_2 concentration dependence are shown in Figure 3. The concentration plots followed to a linear function

$$R_i = r_i C + A \quad (3)$$

where $R_i = 1/T_i$, C – MNPs concentration in mM, r_i – molar relaxivity, and A – a constant determined by the rate of relaxation of water protons in the absence of paramagnetic entity in solution, $i = 1, 2$.

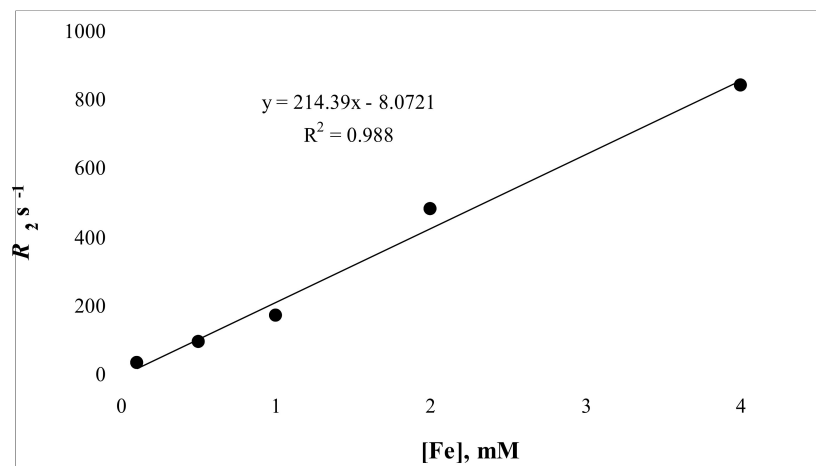
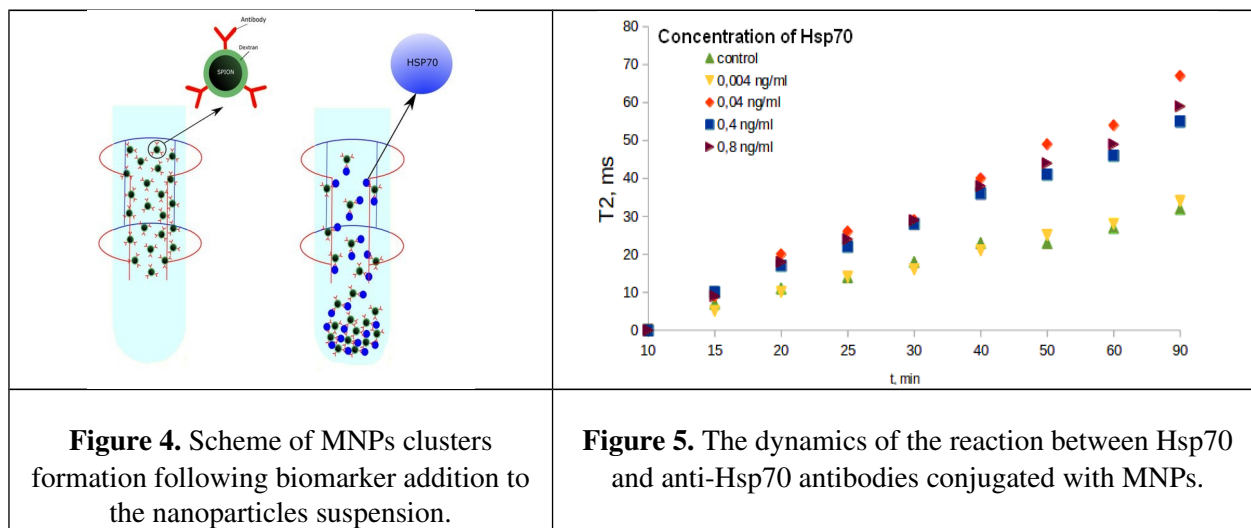


Figure 3. Magnetic relaxation rate R_2 of magnetic conjugate in dependence on the Fe content in MNPs.

According to the relations of outer-sphere magnetic relaxation theory (2, 3) the magnetic relaxation rates increase with growth of MNPs diameter in initial stages of aggregation provoked by antibody-antigen interaction. Magnetic field enhanced targeted aggregation is the key principle of switching assay. The decreasing number of MNPs due to the formation of magnetic clusters leads to creation of motional averaging of local magnetic fields with subsequent increase of estimated relaxation time T_2 . Addition of biomarker to the suspension of magnetic conjugates led to the formation of MNPs clusters that further precipitated from the detection area of NMR spectrometer and thus increased T_2 relaxation time as shown in Figure 4 and Figure 5.



The Figure 4 in schematic form illustrates the influence of antigen-induced aggregation on detected T_2 relaxation of water protons in NMR sample. The observed tendency of increased T_2 relaxation time (Figure 5) indicates the predominant mechanism of motional averaging at analyte concentrations in the analyzed samples. The inhomogeneous contribution of large clusters is suppressed by their sedimentation in the suspension and leaving from the detection coil. The biosensing reaction was observed in the wide range of biomarker concentrations (0.004 - 0.8 ng/ml of Hsp70, 0.4 - 4 ng/ml of antibodies) that is significantly higher than that of the widely applied enzyme-linked immunosorbent assay (ELISA). It is worth noting the fact that standard ELISA is based on the absorption spectroscopy that limits its accuracy in biological fluids, while switching assay allows to overcome this limit due to independency on the scattering centers in media.

4. Conclusions

The obtained data clearly demonstrate the feasibility to apply MNPs conjugated with anti-Hsp70 antibodies for Hsp70 detection in the switching assay. Formation of MNPs clusters due to the interaction with the soluble protein significantly affects T_2 relaxation time of water protons thus enabling to detect the Hsp70 with high sensitivity in comparison to standard diagnostic tools (i.e., ELISA).

Acknowledgments

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References

- [1] Min C, Shao H, Liong M, Yoon T, Weissleder R, Lee H 2012 *ACS Nano* **6** 6821
- [2] Swierczewska M, Liu G, Lee S, Chen X 2012 *Chem. Soc. Rev.* **41** 2641
- [3] Shevtsov M, Yakovleva L, Nikolaev B, Marchenko Y, Dobrodumov A, Onokhin K, Selkov S, Mikhrina A, Guzhova I, Martynova M, Bystrova O, Ischenko A, Margulis B 2014 *Neuro-Oncology* **16** 38
- [4] Vetchinin S, Belova E, Baranov A, Sapozhnikov A, Margulis B Patent 2 381 271 C1 Russian Federation 2010
- [5] Haan H 2011 *Magnetic Resonance in Medicine* **66** 1748
- [6] Roch A, Gossuin Y, Muller R, Gillis P 2005 *J Magn Magn Mater* **293** 532