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BSA hydrogels for EPR spin-labeled anticancer drug delivery

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Introduction

Due to its intrinsic physiological function to bind and transport various endogenous and exogenous substances, including hydrophobic drugs, the protein serum albumin (SA) has been shown to be an excellent choice of polymer for the design of biocompatible hydrogels for controlled and targeted drug release. SA increases drug solubility in blood plasma, prolongs *in vivo* half-life, thereby increasing the therapeutic efficacy of the drug. Several types of SA hydrogels obtained by heat/pH-induced conformational changes, disulfide linking, or by the addition of cross-linkers, have been prepared to date, from bovine and human SA (BSA, HSA), with the aim to synthesize robust hydrogels with retained physiological functions of the protein. The objective of this study was to investigate drug release kinetics from an anticancer drug-depot SA hydrogel, ultimately intended for molecular imaging of drug treatment response.

Experimental Methods

The spin-labeled cytotoxic ligand (**HL**) was synthesized as reported previously [1]. The hydrogels were prepared from BSA (Sigma). Electron paramagnetic resonance spectroscopy (EPR) was performed on a Bruker Biospin Elexsys II E540 EPR spectrometer. The molecular docking (MD) simulations were performed using Autodock software.

Results and Discussion

The binding and release of a highly cytotoxic modified paullone ligand bearing a TEMPO free-radical (**HL**) to BSA was investigated in solution, and in the thermally-synthesized hydrogel by EPR. Namely, drug binding to SA may be monitored by EPR when the drugs are spin-labeled with an “EPR-active” moiety, such as TEMPO. It has been previously shown that **HL**, which belongs to a class of indolo[3,2-d]benzazepines, besides inhibiting cyclin-dependent kinase and glycogen synthase kinase-3, exhibits high antiproliferative activity in human cancer cell lines, also targeting human R2 ribonucleotide reductase protein. The results show that **HL** binds to BSA in solution (Fig 1a), and that the binding is not affected by the heat-induced conformational change of the protein during hydrogel synthesis. Based on the EPR spectral parameters, it appears that **HL** binds to two different binding sites in BSA, within which **HL** is more or less immobilized. By comparison with the EPR spectrum of TEMPO incubated with BSA (Fig 1b), it is concluded that the binding of **HL** to BSA does not occur through the free radical moiety, but rather through the paullone backbone. Furthermore, **HL** is not released from the hydrogel during dialysis in NaCl physiological solution at room temperature for 48 h. After 72 h, only the strongly bound **HL** is detected in the hydrogel. This suggests that the SA hydrogel is a suitable drug-depot, since the water uptake during the 48 h swelling process leads only to the weakly bound ligand displacement. Additional ligand release is likely to be determined by the rate of the *in vivo* gel biodegradation, which certainly may be fine-tuned by varying the physicochemical properties of the hydrogel. The binding was confirmed by MD simulations which showed that **HL** binds to one BSA site with high affinity (Fig 1c).

Conclusion

This work shows that EPR is suitable for SA hydrogel drug release studies, with some advantages over the typically used methods (ultracentrifugation, UV/vis spectrophotometry, fluorescence, Raman spectroscopy), such as the requirement of extremely small volumes of protein (20 µl), sensitivity to minor changes in the spin-label environment (protein conformational changes), nanomolar detection limit, as well as time efficiency. The described methodology,

involving EPR spin-labeling and MD simulations, may give insight into the extent of drug binding and the affinity of the drug for SA, which is of essential importance for controlled drug release assessment.

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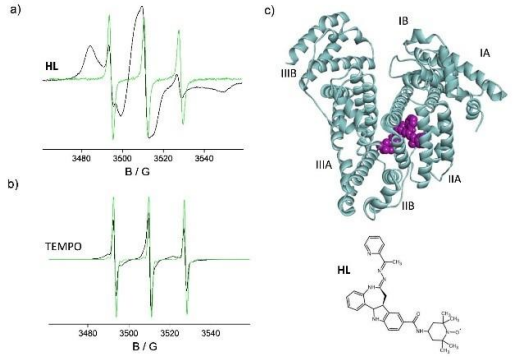

Spin-labeled anticancer drug binding to BSA
Figure 1. EPR spectra of a) **HL**, and b) TEMPO in water (green), and bound to 30 *wt%* BSA (black). Experimental parameters: microwave frequency 9.85 GHz, microwave power 10 mW, modulation frequency 100 kHz, modulation amplitude 1 G; c) 3D representation of **HL** interaction with BSA (PDB ID: 4or0). Starting geometry of **HL** was constructed using Avogadro software and further treated by energy minimization using density functional theory until reaching all positive Hessian matrix eigenvalues. Intermolecular interactions were simulated using Autodock.

References

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