MR imaging of protein folding employing Nuclear–Overhauser–mediated saturation transfer

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Target audience: Researchers and clinicians who are interested in protein folding and the mechanism of *in vivo* CEST-MRI.

Purpose

The biological function of proteins is governed by their tertiary structure. NMR spectroscopy can be used to obtain protein structures by studying dipolar interactions between intramolecular protons known as Nuclear-Overhauser-enhancement (NOE) effects[1]. Recent studies observed an NOE-mediated saturation transfer between aliphatic protons and the large water pool which can be employed analogue to chemical exchange saturation transfer (CEST) MR imaging [2,3]. This work tests the hypothesis that the aliphatic NOE-MRI signal is a function of protein structure, besides its relation to protein concentration. It is further discussed if the observed correlation might play a role for MRI of brain tumors and necrosis where a drop of the NOE signal is observed[2,4,5].

Methods

Seven 20 ml tubes with 2.5 % BSA in phosphorus–based sodium–potassium buffer contained increasing concentrations of urea from 0.0 M to 7.0 M (Fig 1a). Fluorescence was monitored at an emission wavelength of 335 nm, exciting selectively the two tryptophan residues of BSA at λ= 295 nm. Pixelwise Z-spectra of BSA solutions (3%, PBS) with different urea concentrations (0M-8M) were acquired at a Siemens whole–body MR B_0=7T by a 2D-GRE sequence. Presaturation consisted of 400 Gauss pulses (t_p = 10ms, t_d = 20 ms, DC = 30 %, B_1 = 1.2 µT from –6 ppm to 6 ppm in steps of 0.2 ppm. *In vivo* a 3D-GRE readout[6] was used with presaturation: 5 pulses (100ms, B1=0.7µT). For evaluation MTR=1-Z(-3ppm) or MTR_{asym}=Z(-3ppm)-Z(+3ppm) was used, where Z= S_{sat}/S_0.

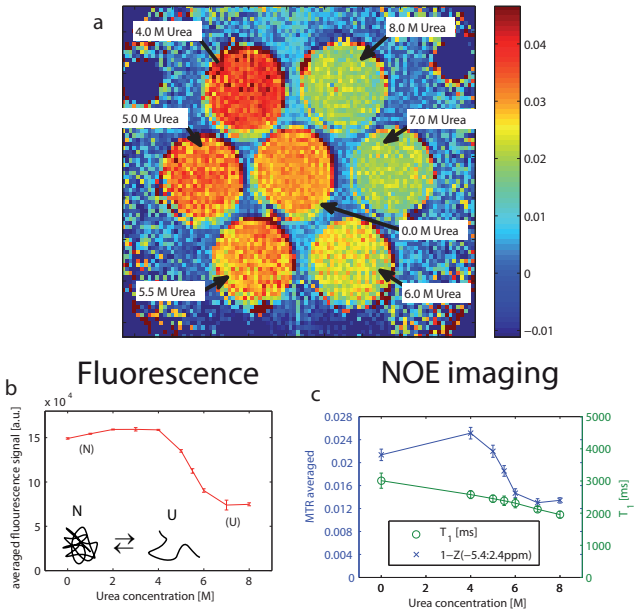

Figure 1: NMR imaging of protein unfolding of BSA (a). The NOE imaging signal observed by MTR(–3 ppm) decreases with higher urea concentration which indicates loss of protein tertiary structure. Urea–dependent unfolding of BSA, monitored by fluorescence spectroscopy (b) shows high correlation with the signature of NOE imaging (c).

Results

Urea-dependent unfolding of BSA, which was monitored by fluorescence spectroscopy, clearly affects the aliphatic NOE imaging contrast (Fig 1a). The ROI-averaged NOE effect (Fig 1c) shows the same sigmoidal signature as observed by fluorescence (Fig 1b), which cannot be explained by the linear decrease of T_1 (green line, Fig 1b).

Discussion

The NOE is a function of interaction distances between spins, and correlation times. Both parameters strongly depend on the structure of the protein, which–is strongly changed by unfolding. Amide proton transfer could not be studied as the urea amine resonance overlapps with this spectral region. Figure 2 shows that NOE signals in tumor areas are decreased compared to normal tissue. This was observed in 10 glioblastoma patients (two shown in Fig 2). Three explanations are considered: reduced protein content in the voxel[2,7], lower restriction and thus larger tumbling of whole proteins, or protein unfolding. Until now we have no further evidence which pathway is dominant. However, we think that protein unfolding might play a dominant role in the necrosis.

Conclusion

Our hypothesis that the NOE signal is a function of protein structure could be verified and protein folding should be considered as an additional property affecting saturation transfer between water and proteins besides the microenvironment and physiological quantities like metabolite concentration, temperature, and pH. Our observation also points to a possible contrast mechanism for CEST-MRI, sensitive to the structural integrity of proteins in cells.

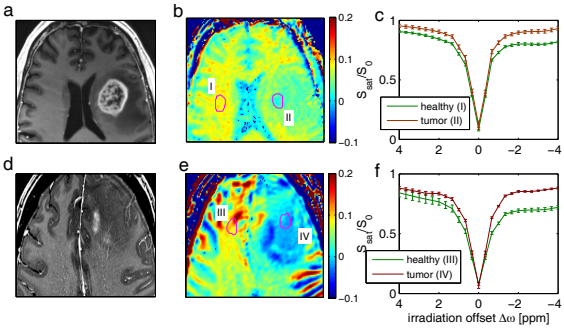

Figure 2: Gd–enhanced T_1–weighted images show enhancement in tumor regions of patient #1 (a, b, c) and patient #2 (d, e, f). NOE measured by MTR_{asym}(-3ppm) (b, e) is reduced in tumor tissue. Both images exhibit a decrease of MTR_{asym} within the tumor area.

References

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