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Relaxivities of human liver and spleen ferritin

Yves Gossuin[a,*], Robert N. Muller[b], Pierre Gillis[a], Lionel Bartel[a]

[a]*Biological Physics Department, University of Mons-Hainaut, 7000 Mons, Belgium*

[b]*NMR and Molecular Imaging Laboratory, Department of General, Organic and Biomedical Chemistry, University of Mons-Hainaut, 7000 Mons, Belgium*

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Abstract

Ferritin, the iron-storing protein of mammals, is known to darken T_2-weighted magnetic resonance images. This darkening can be used to noninvasively measure an organ's iron content. Significant discrepancies exist between T_2 data obtained with ferritin-containing tissues and with aqueous solutions of horse spleen ferritin (HSF). The NMR properties of stable human ferritin have never been studied in aqueous solutions. Relaxometry results on human liver and spleen ferritin are reported here, showing that the relaxation induced in aqueous solutions by human ferritins is comparable to that induced by HSF. As a consequence, the differences between ferritin-containing human tissues and ferritin solutions cannot be attributed to different NMR properties of human and horse ferritins, but probably to a clustering of the protein in vivo.

Keywords: Ferritin; Liver; Spleen; Relaxation times; Iron content of tissues

1. Introduction

Magnetic resonance imaging (MRI) was proposed early on for the in vivo quantification of ferritin-bound iron in the liver, spleen and brain [1,2]. Various MRI protocols have since been used to study the distribution of ferritin in the liver [3–12] and in the brain, especially in the cases of Parkinson's and Alzheimer's diseases [13–19]. Indeed, ferritin, the mammal's iron storage protein, contains a superparamagnetic ferrihydrite ($5Fe_2O_3$–$9H_2O$) crystal [20,21] that accelerates the transverse relaxation of water and therefore darkens T_2-weighted images. For a better understanding of the MRI contrast caused by ferritin, numerous studies have investigated the relaxation of aqueous solutions of horse spleen ferritin (HSF) and hydrated iron oxide nanoparticles [22–29], finally showing that ferritin-induced T_2 shortening arises from the binding of water protons to the surface of the ferrihydrite crystal. This unique relaxation mechanism is responsible for the unusual proportionality between $1/T_2$ and the applied magnetic field observed in solution [24,25,27] and in tissues [26,30–32]. The influence of ferritin on in vivo MRI contrast will therefore grow together with the increase of the imaging magnetic fields, as does $1/T_2$. Indeed, $1/T_2$ of a ferritin-containing brain tissue will be about two to three times larger in a 3-T MR scanner than in a 1-T machine [33].

For almost all the MRI protocols of iron content evaluation, the general qualitative correlation between the measured parameter ($1/T_2$, $1/T_2$*, etc.) and the iron concentration

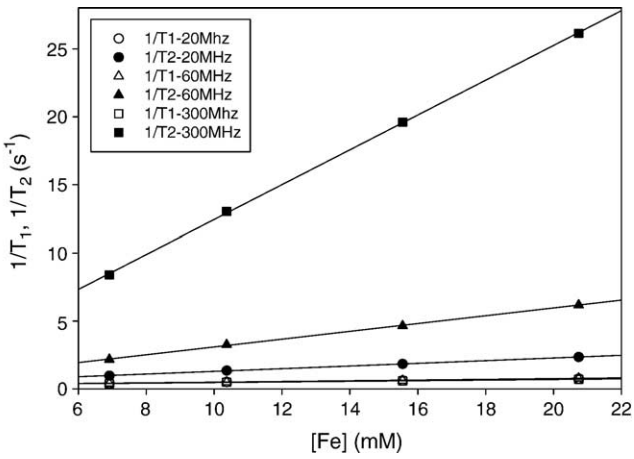

Fig. 1. Evolution of $1/T_1$ and $1/T_2$ with iron concentration for human spleen ferritin at 20, 60 and 300 MHz.

* Corresponding author. Service de Physique Expérimentale et Biologique, Faculté de Médecine, Université de Mons-Hainaut, 7000 Mons, Belgium. Tel.: +32 65 373537; fax: +32 65 373537.

E-mail address: yves.gossuin@umh.ac.be (Y. Gossuin).

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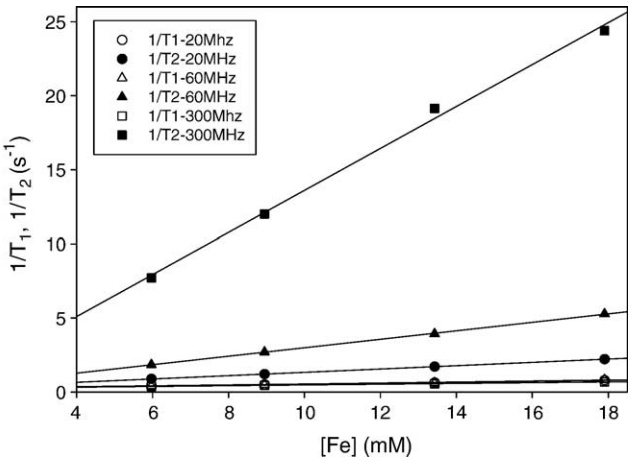

Fig. 2. Evolution of $1/T_1$ and $1/T_2$ with iron concentration for human liver ferritin at 20, 60 and 300 MHz.

was checked, but the measurements seem to be too sensitive to the experimental procedures and to physiological data (type of organ, iron content, degradation of the tissues, etc.) to allow for their general use in hospitals [34].

These difficulties are clearly related to the unexplained yet significant differences between ferritin-induced relaxation in aqueous solutions and in tissues: for the same iron concentration, at 1 T, the transverse relaxation rate is more than three times greater in tissue than in HSF aqueous solution. Even in vivo, the rate is significantly greater in mouse liver than in spleen [32]. The reasons for these differences could be:

- Structural differences between HSF (used for the aqueous solution studies) and human ferritin.
- In vivo clustering of ferritin, affecting transverse relaxation properties, which has been shown to depend on the type of organ [35–37]. It should be noted that the restriction of water diffusion in tissues could also contribute, in part, to the relaxivity differences.

To discriminate between these hypotheses, the relaxation properties of stable, nonclustered human ferritin were studied. More particularly, the longitudinal (r_1) and transverse (r_2) relaxivities (i.e., the increase of relaxation rate induced by an increase of 1 mmol in the iron concentration) were determined and compared to HSF data.

If these relaxivities are comparable to those obtained for HSF, it is an indication that the clustering of the protein could be the only reason for the increase of T_2 shortening observed in tissues.

2. Materials and methods

2.1. Samples

Human liver and spleen ferritin samples were obtained from Scipac (Sittingbourne, UK). The purity of the sample was better than 96%. The average loading factor (number of iron ions per molecule) of the ferritin samples, determined from the iron mass fraction, was 1740 and 2740 for the liver and spleen ferritin, respectively. The hydrodynamic size of the protein, as measured by photon correlation spectroscopy (BIC-9000, Brookhaven Instruments, Holtsville, NY, USA), was 17 and 12 nm for the liver and spleen ferritin, respectively. These results show that the samples are stable and do not present important clustering.

Relaxation time measurements were performed on BRUKER PC110, PC120, PC140 and mq 60 instruments working at proton Larmor frequencies (v_0) of 10, 20, 40 and 60 MHz, respectively (a magnetic field of 1 T corresponds to a proton Larmor frequency of 42.6 MHz). A BRUKER AMX 300 (300 MHz) spectrometer was used for the high-field measurements. T_2 was obtained at 37°C with a Carr–Purcell–Meiboom–Gill sequence, with a TE of 1 ms. The repetition time was always longer than 5 T_1. The mono-exponential fits were good, thereby providing no evidence of a multiexponential behaviour. The error on the relaxation times was less than 4%.

3. Results and discussion

Figs. 1 and 2 show T_1 and T_2 data for human spleen and liver ferritin, respectively. As expected, $1/T_1$ and $1/T_2$ increase linearly with the iron concentration, and the slope of this increase gives the longitudinal and transverse relaxivities of ferritin. The relaxivities obtained are approximately the same as for HSF, but clearly smaller than those obtained in tissues (Table 1). Moreover, $1/T_2$ increases linearly with magnetic field for both samples, as previously observed in solutions of HSF and ferritin-containing tissues (Fig. 3). The normalized slopes (α) of this linear relationship between $1/T_2$ and Larmor frequency ($\alpha = 4.41\times10^{-3}$ s^{-1} MHz^{-1} mM^{-1} for liver ferritin and $\alpha = 4.07\times10^{-3}$ s^{-1}

Table 1
Relaxivities of human spleen and liver ferritins

	20 MHz	60 MHz	300 MHz
Human spleen ferritin	$r_2 = 0.099$ s^{-1} mM^{-1}	$r_2 = 0.287$ s^{-1} mM^{-1}	$r_2 = 1.28$ s^{-1} mM^{-1}
	$r_1 = 0.0238$ s^{-1} mM^{-1}	$r_1 = 0.0261$ s^{-1} mM^{-1}	$r_1 = 0.0224$ s^{-1} mM^{-1}
Human liver ferritin	$r_2 = 0.112$ s^{-1} mM^{-1}	$r_2 = 0.287$ s^{-1} mM^{-1}	$r_2 = 1.42$ s^{-1} mM^{-1}
	$r_1 = 0.0303$ s^{-1} mM^{-1}	$r_1 = 0.0338$ s^{-1} mM^{-1}	$r_1 = 0.0259$ s^{-1} mM^{-1}
Horse spleen ferritin [27]	$r_2 = 0.127$ s^{-1} mM^{-1}	$r_2 = 0.283$ s^{-1} mM^{-1}	$r_2 = 1.22$ s^{-1} mM^{-1}
Ferritin in human liver [7]	At ~20 MHz, $r_2 = 0.331$ s^{-1} mM^{-1}		
Ferritin in primate liver [30]	At ~40 MHz, $r_2 = 0.69$ s^{-1} mM^{-1}		

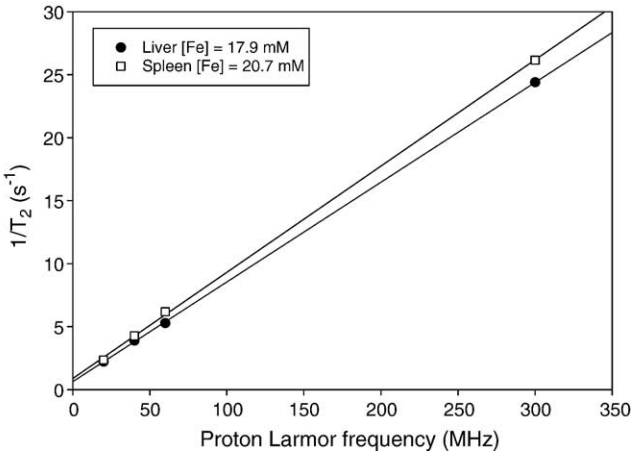

Fig. 3. Evolution of $1/T_2$ with magnetic field for human liver and spleen ferritin.

MHz^{-1} mM^{-1} for spleen ferritin) are consistent with the value obtained for HSF solution ($\alpha = 3.92\times10^{-3}$ s^{-1} MHz^{-1} mM^{-1}), but clearly below the slope measured in ferritin-containing tissues, for example, in the brain's globus pallidus ($\alpha = 8.4\times10^{-3}$ s^{-1} MHz^{-1} mM^{-1}) [26] and in angioma of the brain ($\alpha = 21.2\times10^{-3}$ s^{-1} MHz^{-1} mM^{-1}) [38].

These results indicate that human and HSFs have similar NMR behaviors. Thus, the significant differences observed between ferritin in solutions and in tissues seem to be clearly related to the clustering of the protein in tissues, a clustering that is not observed using stable aqueous solutions of ferritin and that may depend in vivo on the type of organ. This is consistent with recently published results showing that in mouse spleen, where clustering is not significant, the T_2 behavior is similar to that in HSF solutions, while in mouse liver the T_2 shortening is clearly greater [32].

A first indication in that direction was given by the study of Wood et al. [39] showing an increase of transverse relaxivity in liposomal ferritin. This r_2 increase, which was shown to depend on the echo time, could be due to the accumulation of fields from all the particles in an aggregate. It is possible that at a certain stage in the clustering process, the diffusive part of relaxation — the outer sphere contribution, clearly dependent on the echo time — becomes significant and also contributes to the transverse relaxivity. However, this contribution should be clearly identified by a quadratic increase of $1/T_2$ with magnetic field, which has never been observed for ferritin, neither in solution nor in tissues. An on-going in vitro experimental study of ferritin clustering, consistent with the previous interpretation, should soon provide the final piece of the puzzle for the complete understanding of ferritin-induced relaxation in solutions and in tissues. This understanding will help to establish the best conditions to obtain a good correlation between MRI contrast and iron content. High fields are clearly better, but what sequence should be used? That question remains to be answered.

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