

Electron transfer in ferritin as probed by muon spin relaxation

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1. Introduction

Ferritin is the normal iron storage protein found in plants, bacteria and animals. The ferritin molecule is composed of an 8 nm diameter iron-based, ferrihydrite-like core of up to 4500 Fe (III) ions, inside a spherical protein shell. The shell (~2 nm thick) is composed of 24 polypeptide chains [1]. The structural [2] and magnetic [3] properties of ferritin have been the subject of much investigation. The conducting properties of the protein shell are now under scrutiny. Measurements have shown that the shell may act as an electron conductor [4]. As a result, the possibility that ferritin could be used in a bio-nano-battery is extremely topical [5, 6]. Muon spin relaxation (μ SR) is one experimental technique that allows characterization of the transport properties of a system. μ SR is a microscopic probe which measures the time dependent spin dynamics of an excitation. It has an advantage over other techniques (i.e. ESR) since it both generates the excitation and then acts as a probe of the dynamical properties of the excitation. The technique is well established and described in detail in [7]. Longitudinal field muon spin relaxation (LF- μ SR) has already been used to study muoniated radical formation [8] and superparamagnetism in ferritin [9] as well as electron transport in apoferritin [10]. However, as far as we are aware, the technique has not been used to study electron transfer mechanisms in ferritin. In this paper we present the results of a LF- μ SR study of electron transfer in a sample of lyophilized horse spleen ferritin ($80 < T < 280$ K) using the labeled electron method.

2. Muon Spin Relaxation

When positive muons, μ^+ , are deposited in a chemical sample at least three possible events can occur:

- (i) μ^+ sits in the sample and decays (characteristic lifetime = 2.2 μ s)
- (ii) μ^+ combines with an electron to form a muonium atom, Mu, a radioactive light isotope of hydrogen.
- (iii) Mu reacts with the substrate to form a muonium-substituted radical, or resides in a diamagnetic environment.

Any resulting positron emission will occur preferentially in the spin direction according to, $W_o = 1 + a_o \cos \theta$ (θ is the angle between the spin and direction of positron emission). In (i) the muons retain their full spin polarisation. However, if a muonium atom is formed, as in (ii), 50% of the spin polarisation is lost; this deficit is frequently referred to as the 'missing fraction' and arises from depolarization of the muonium signal. This polarisation can be restored by the application of a magnetic field along the direction of the spin polarisation. The muon and electron spins become decoupled by the applied magnetic field and polarisation is restored. All of these events can be detected and characterised using either the ZF- μ SR or LF- μ SR technique.

3. The Risch-Kerr Model [11]

Risch and Kehr [11] (R-K) considered the spin relaxation of a muon interacting with a spin defect rapidly diffusing along a one-dimensional (1D) chain. Here the LF muon spin relaxation function, $G_z(t)$, has the form,

$$G_z(t) = \exp[\Gamma(B)t] \operatorname{erfc}\{[\Gamma(B)t]^{0.5}\} \quad (1)$$

for $\lambda t_{\max} \gg 1$. erfc signifies the complementary error function, $\Gamma(B)$ is a magnetic field dependant R-K relaxation parameter, λ is the electron spin rate and t_{\max} is the experimental timescale. In finite magnetic fields $\Gamma(B)$ is given by

$$\Gamma(B) = \lambda / (1 + (2\omega_e \lambda)^{1/2} D_{||} / \omega_0^2)^2 \quad (2)$$

where $\omega_e = \gamma_e B$ ($\gamma_e = 1.76 \times 10^{11}$ HzT⁻¹, the gyro-magnetic ratio of the electron), ω_0 ($= 2\pi A$) is the μ -electron hyperfine coupling frequency and $D_{||}$ is the intra-chain, or 1D, diffusion rate. ω_0 is obtained from the longitudinal

decoupling field, B_o , of the initial muon asymmetry. For a detailed discussion of how B_o is determined see Kilcoyne and Webster [8]. In the fast diffusion limit the intra-chain diffusion can be determined from the expression,

$$D_{\parallel}^2 \gg \omega_o^4 / 2\omega_e \lambda \quad (3)$$

As the field is reduced, however, a cut off in the inverse field dependence is predicted and $\Gamma(B)$ becomes field independent. The critical field at which this cut off occurs is the field at which ω_c becomes smaller than the inter-chain (D_{\perp}) or 3D, diffusion rate. An estimate of D_{\perp} can be obtained by assuming that the field dependent behavior of Γ has the form, $\Gamma(B) = \Gamma_o / (1 + (B/B_c))$; B_c is the critical field above which $\Gamma(B)$ evolves from field-independent to field dependent. D_{\perp} can be determined using $D_{\perp} = \gamma_e B_c$. The R-K model of spin relaxation has been applied to studies of electron motion in conducting/non-conducting polymers [12] and in proteins and DNA [13].

4. Experimental Details

ZF- μ SR measurements were collected using the ARGUS instrument (RIKEN-RAL Muon Facility, UK [14]). Lyophilized horse spleen ferritin (1 gram, Fe(3+), from Sigma Chemicals, UK) was wrapped in thin silver foil and cooled using a helium cryostat. Polarisation decay spectra were collected between 0.1 to 16 μ s in both ZF and longitudinal magnetic fields ($50 < B_{\text{ext}} < 3800$ Gauss) and at temperatures between 15 and 280 K. Field dependent room temperature data were collected for calibration purposes from a pure (99.99%) silver sample and a quartz plate.

5. Results and Discussion

Examples of the μ SR relaxation spectra and fits are shown in Fig. 1(a). Above 50 G, the data is best fitted using the R-K model, modified to include a temperature and time-independent background term, A_{bck} . The magnitude of A_{bck} (3.51 %) was obtained by fitting the 15 K ZF data to $G_z(t) = a_o \exp(-\lambda t)^{\beta} + A_{\text{bck}}$. Previous [15] measurements on lyophilized ferritin show that the system behaves superparamagnetically, with a frequency dependent blocking temperature (T_b), between 15 and 50 K. Indeed, ZF- μ SR ferritin studies by Cristofolini *et al* [9] show a change in spin relaxation between 11 and 60 K; interpreted as a freezing of the superparamagnetic moments in the individual iron oxide cores. In order to avoid complications associated with spin freezing we consider here only data collected above T_b as seen by μ SR (i.e. 60 K). The analysis of data below 60 K will be the subject of a future publication.

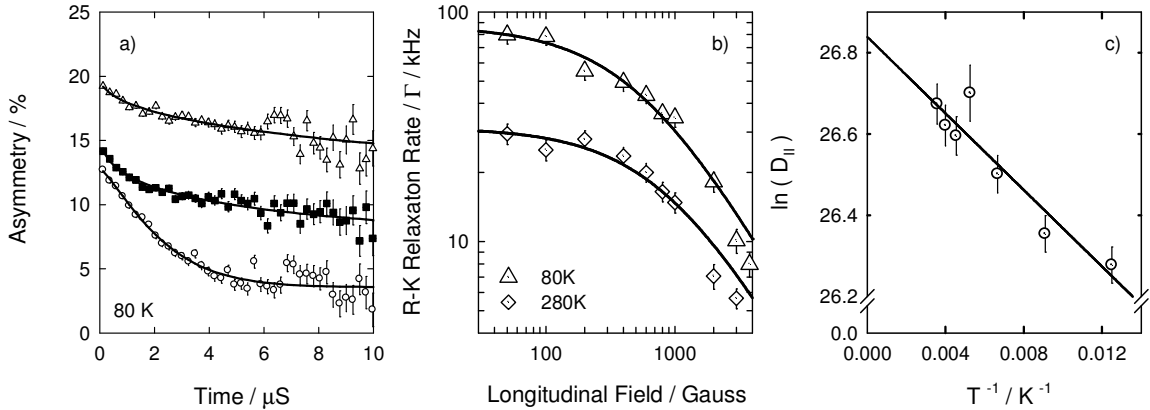


Fig. 1(a) Typical μ SR spectra and fits to the R-K model collected at 80 K and in 0 G (o), 50 G (■) and 3000 G (Δ). b) The field dependence of the R-K relaxation parameter, $\Gamma(B)$, at 80 and 280 K. c) Temperature dependence of the intra-chain diffusion rate, $D_{\parallel}(1\text{kG}, T)$ as determined from Eq. 2. The solid lines in each plot are fits to the data using the functions described in the text.

Using the analysis model outlined above, values of the R-K relaxation parameter, Γ , were determined from spectra collected above 60 K and at fields above 50 Gauss. The field dependence of $\Gamma(B)$ at 80 and 280 K is illustrated in Fig. 1(b). The solid lines in (b) are the results of fitting $\Gamma(B) = \Gamma_o / (1 + (B/B_c))$ to the data. For $B_{\text{ext}} <$

400 G, $\Gamma(B)$ is weakly field dependent. As the field increases, however, $\Gamma(B)$ becomes inversely proportional to B_{ext} at some critical field, B_c . For ferritin, B_c shows no appreciable temperature dependence and a mean cut-off field value of 475 +/- 54 Gauss has been determined for ferritin between 80 and 280 K. Subsequently, the mean inter-chain diffusion rate, $D_{\perp}(\text{mean})$, derived from B_c via the expression $D_{\perp} = \gamma_c B_c$, was found to be $(8.04 \pm 0.95) \times 10^9 \text{ rad s}^{-1}$. While the magnitude of $D_{\perp}(\text{mean})$ is comparable to that ascertained from labelled electron studies of cytochrome-c and myoglobin [16], the latter both exhibit a marked increase in D_{\perp} with increasing temperature over the temperature range studied here. In addition, while the response of $\Gamma(B)$ at low external fields is comparable to that observed from the protein cytochrome-c (Fe^{3+} type), it is markedly different to that observed from samples of DNA with specific humidity, water molecule concentration and base pair configurations [13]. Surprisingly in apoferritin [10] $\Gamma(B)$ is also field dependent at all fields and all temperatures studied and therefore no value of D_{\perp} can be determined via this model.

Above 600 Gauss $\Gamma(B)$ is inversely proportional to B_{ext} at all measured temperatures. The R-K model predicts such behavior is indicative of intra-chain diffusion. It should be noted that Risch and Kehr developed their model by considering a 1D chain. This is not the situation here. Each of the 24 protein subunits in ferritin is composed of a folded bundle of helically twisted segments. As a consequence, the possibility of intra-chain diffusion between loops may be indistinguishable from inter-chain diffusion and cannot be ruled out. The temperature dependence of, D_{\parallel} , determined by fitting the high field $\Gamma(1 \text{ kG}, T)$ values to Eq. 2 is shown in Fig. 1(c). 1 kG has no particular significance but allows us to draw comparisons between this work and that reported by other authors. D_{\parallel} is seen to increase with increasing temperature, although diffusion rates of the order $10^{11} \text{ rad s}^{-1}$ are obtained at all temperatures. Again, this rate is comparable to D_{\parallel} reported for myoglobin and cytochrome-c [16]. However, unlike ferritin, D_{\parallel} determined from myoglobin decreases between 100 K and 280 K. In contrast, D_{\parallel} for cytochrome-c shows a broad peak between 100 and 200 K and decreases as the temperature is elevated further. In apoferritin D_{\parallel} is of the order $10^{12} \text{ rad s}^{-1}$, but decreases smoothly as the temperature is increased [10]. Since the protein shells of ferritin and apoferritin are equivalent, our results suggest that the presence of an iron core influences the electron transfer process. $D_{\parallel}(1 \text{ kG}, T)$ between 80 and 280 K is well represented by the Arrhenius form suggesting a thermally activated process, at least down to 80 K. Assuming that $D_{\parallel}(1 \text{ kG}, T) = D_{\parallel}(1 \text{ kG}, 0) \exp(-E_a / kT)$, where E_a is an activation energy and k is the Boltzman constant, a plot of $\ln(D_{\parallel})$ against T^{-1} , (see Fig 1(c)) gives the effective activation energy $E_a = 3.9 \pm 0.7 \text{ meV}$.

6. Conclusions

We have used LF- μ SR to investigate electron-transfer processes in ferritin with a $\text{Fe}(3+)$ core. Data collected at finite fields, and above T_b (i.e. 60 K), is well described using the R-K model at all measured temperatures. For $B_{\text{ext}} < 400 \text{ G}$, the R-K relaxation parameter, $\Gamma(B)$, shows little field or temperature dependence. This response is indicative of inter-chain diffusion with inter-chain diffusion rates of the order 10^9 rad s^{-1} observed between 60 and 280 K. Between 600 and 3800 G, the R-K relaxation parameter is inversely proportional to applied field. Such behavior is predicted by R-K as indicative of intra-chain diffusion. Intra-chain diffusion rates of the order $10^{11} \text{ rad s}^{-1}$ have been determined for $B_{\text{ext}} = 1 \text{ kG}$, with the diffusion rate increasing as the temperature is elevated. The temperature dependence of $D_{\parallel}(1\text{kG}, T)$ follows an Arrhenius form with a characteristic activation energy of $E_a = 3.9 \pm 0.7 \text{ meV}$.

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