Muonium response to oxygen content in biological aqueous solutions for cancer research

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Abstract. Muonium (Mu), which is known to exhibit a characteristic concentration dependent spin relaxation change when impacting molecular oxygen dissolved in water, was found to show a similar behaviour in aqueous solutions of Tris Buffered Saline (TBS), albumin, serum, and hemoglobin (Hb). These effects, along with the interaction of Mu with deoxy-Hb (which is modulated by oxygen by the formation of oxy-Hb) suggest that the muon method can be applied to systematic studies of oxygen dependent effects in biological systems. This muon method may particularly be applicable at low (hypoxic) oxygen levels, an important consideration in the radiation treatment of cancer.

1. Introduction

Hypoxia, or low oxygenation, is known as an important factor in tumor biology and the response of tumors to radiation treatment. In cancer patients, an accurate measurement of hypoxia in specific regions may have an important predictive value in the management of treatment and outcome of the disease [1, 2, 3, 4]. The National Cancer Workshop (Tatum, et al 2006 [4]) on hypoxia imaging techniques pointed out the need for improved O₂ detection methods for cancer treatment. Here, we propose the use of the polarized positive muon (µ⁺) produced at the accelerator facility as a new sensitive method to probe existence of the paramagnetic O₂ in the cancer of eventually human body.

There have been trials employing PET, MRI and EPR for this purpose but with major limitations as summarized in the followings [4]. PET ¹⁸F-labelled fluoro-misonidazole (¹⁸F-FMISO) tracer is widely used in PET for monitoring hypoxia. It does not directly measure molecular O₂ in the tumor, but the tracer retention affected primarily by O₂. It images hypoxic cells and re-oxygenation following radiation therapy, which is usually measured in off-line manner after radiation therapy.
MRI Blood oxygen level-dependent (BOLD) MRI does not directly measure $O_2$ molecule in blood but rather detects deoxy-Hb. Also, special attention is required for use of the high magnetic fields for in-situ MRI.

EPR Two unpaired electrons in molecular $O_2$ cause spin relaxation of the surrounding paramagnetic constituents. Usually, infusible tracers are used to detect line width broadening due to interactions with molecular $O_2$. Thus, the EPR method is not truly non-invasive.

On the other hand, since late 70s, there have been experimental studies on the effects of dissolved oxygen on the spin relaxation of Mu in pure water [5, 6, 7]. The relaxation rate constant change $\lambda_{Mu}/[O_2]$ of Mu is known to be $(1.8 \pm 0.1) \times 10^{10}$ L mol$^{-1}$ s$^{-1}$ [7]. The origin of this relaxation is ascribed to electron spin exchange interaction with dissolved paramagnetic molecular $O_2$. The sensitivity range to the spin relaxation of a pulsed muon is $10^4$ to $10^7$ s$^{-1}$ for an intense muon beam now available at accelerator laboratories like ISIS (UK) or J-PARC (Japan). Therefore, the sensitivity of the muon spin probes for $pO_2$ (Mu) becomes $0.5 \times 10^{-6}$ to $0.5 \times 10^{-3}$ mol L$^{-1}$ based on the experimental data mentioned above.

The solubility of $O_2$ in water at 23 $^\circ$C is known as 1.29 $\times$ 10$^{-5}$ mol L$^{-1}$ kPa$^{-1}$ [8]. Thus, under 1 atmosphere, the oxygen solubility $pO_2$(s.l.) at 23 $^\circ$C is $1.3 \times 10^{-3}$ mol L$^{-1}$. (Here s.l. is used for solubility limit.) This means that the measurable partial pressure, which is $pO_2$(Mu) divided by $pO_2$(s.l.) becomes $0.4 \times 10^{-3}$ to 0.4. These values can be expressed in units of mmHg so that the oxygen tension in water sensed by the muon spin probe becomes $3.0 \times 10^{-1}$ to $3.0 \times 10^2$ mmHg perfectly matching to the hypoxia range of values.

The proposed Mu relaxation method is able to detect and measure molecular $O_2$ concentration in tissues directly. It is able to monitor non-invasively in a small area of human tissue at any temperature and without any strong magnetic fields. A particularly desirable feature of the present muon method is a capability of non-invasive and sensitive imaging: spatial resolution of the mm level at the depth of up to 20 cm and $O_2$ concentration sensitivity down to a $pO_2$ of 0.1 mmHg.

The problem to be solved before serious application to hypoxia studies is a possible existence of the background signals from other magnetic molecules in human tissues. Magnetic property of Hb in blood [9] is a concern. At the same time chemical reaction between Mu and biological molecules may disturb the expected observations. In the present study, we conducted several test experiments on Mu spin relaxation $\lambda_{Mu}$ against $O_2$ contents (O2%) in aqueous solution of representative biological molecules. Then, based upon the obtained results, we made predictions for the future development of the muon method. Special emphasis was placed on the Hb aqueous solutions, where by increasing $O_2$ concentration, some fraction of $O_2$ goes to change deoxy-Hb (magnetic) to oxy-Hb (non-magnetic), reducing the Mu spin relaxation rate. Careful measurements were made for $\lambda_{Mu}$ against (c(O2%), c(Hb)).

2. Experimental arrangement

Experiment was conducted at Port 2 of the RIKEN RAL muon facility in UK, by using 60 MeV$/c$ decay-in-flight polarized positive muons.

Once energetic polarized positive muons are injected and stopped in water, it is known for these $\mu^+$ to take electronic states of diamagnetic $\mu^+$ such as $\mu^+OH$ with a fraction of 60%, paramagnetic Mu with a fraction of 20% and a missing fraction of 20% [5, 6, 7]. In the Mu fraction a half becomes ortho state with spin 1, providing a spin rotation signal with 100 times faster precession pattern compared to the diamagnetic $\mu^+$. Spin rotation and its relaxation were detected under 2.1 G transverse magnetic field. All the measurements were conducted at room temperature.

The biological aqueous solutions with controlled $O_2$ concentrations were prepared in a separate flask with magnetically driven stirrer (Fig. 1) and continuously transported into the chamber for muon beam exposure by a circulating flow pump. Oxygen concentration in the gas
space of the flask was monitored by a Vernier Gas monitor and in the liquid phase by a NeoFox oxygen sensor placed inside the muon chamber. The oxygen concentrations of all the data are presented by the reading of the NeoFox monitor. In order to achieve a satisfactorily equilibration between aqueous biological samples and $O_2$ in the gas phase in a short time, Silicone Membrane Modules from Perm Select Co. Ltd. was used. When changing the oxygen concentration ($O_2\%$) in the gas phase, equilibration occurred within 10 min for the 0.7 litre solution in the flask.

The concentration of $O_2$ was controlled from 0% to 20.7% by changing the ratio of $N_2$ gas and air. As a reference, He gas flow was used to justify use of the $N_2$ gas for zero $O_2$ concentration.

### 3. Experimental results

Typical Mu spin rotation spectrum in pure water at various $O_2$ concentrations are presented in Fig. 2. Superimposed on the spin rotation of the diamagnetic $\mu^+$, a clear Mu spin rotation was observed and it shows a characteristic relaxation rate increase with increasing dissolved $O_2$ concentration. Here the $O_2$ concentration was determined by the NeoFox oxygen monitor that was calibrated for $O_2$ concentration at 0% with $N_2$ gas and at 20.7% with air. Note that in the discussion and figures below the oxygen concentrations are provided as percent $O_2$ (e.g. 10%). These values are the gas compositions that would be in the equilibrium with the actual liquid phase concentrations of free molecular oxygen. Thus, 10% $O_2$ at 23 $^\circ$C is equivalent to $1.3 \times 10^{-4}$ mol L$^{-1}$ free molecular oxygen in solution.

The time spectrum is known to be written by the following function,

$$N = N_0 e^{-t/\tau} \left[ 1 + A_\mu \cos(\omega_\mu t + \phi_\mu) + A_{Mu} e^{-\lambda_{Mu} t} \cos(\omega_{Mu} t + \phi_{Mu}) \right] + B,$$

where $N_0$ is a normalization factor, $B$ is the time-independent background and $\tau_\mu$ is the muon lifetime (2.20 $\mu$s). The terms $A_\mu$ and $A_{Mu}$ are the amplitudes of the spin precession corresponding to the polarization asymmetry for the $\mu^+$ in diamagnetic species and in Mu, respectively. The parameter $\lambda_{Mu}$ is the muonium relaxation rate while the relaxation rate of $\mu^+$ in diamagnetic species is assumed to be negligible, $\omega_\mu$ and $\omega_{Mu}$ are the muon and Mu precession frequencies, $\phi_\mu$ and $\phi_{Mu}$ are the respective initial phases of their precessions. Under transverse field of $H(G)$, the spins of $\mu^+$ and Mu take precession with angular velocity of $\omega_\mu(kHz) = 2\pi \times 13.553 \times H(G)$ and $\omega_{Mu} = 2\pi \times 1390 \times H(G)$, respectively. As seen in Fig. 2, the spin precession of the Mu showed faster relaxation with increasing $O_2$ concentration. The data is very consistent with the published data [7].
At first, 7.5 g Tris Buffered Saline (TBS) salt, which is a buffer used in some biochemical techniques to maintain the pH within a relatively narrow range was added to 1 litre of pure water. The Mu spin rotation signal was found to be essentially unchanged. Also, for this TBS solution, Mu was found to show a similar relaxation change with increasing O$_2$ concentration as that for pure water.

Then, 0.4 g albumin was introduced in 1 litre water with 7.5 g TBS buffer and the solution was equilibrated with 10% O$_2$ in the gas phase. Albumin was selected as a typical representative of a biological protein in human tissues. By increasing albumin quantity to 4 g, the Mu signal was found to disappear. In order to observe more clearly, Mu relaxation change with O$_2$ concentration in albumin aqueous solution, systematic measurements were done without TBS. The measurement was extended to 0.5 wt.% serum aqueous solutions. The Mu was found to take a similar relaxation pattern with increasing O$_2$ concentration as previously observed for pure water.

As shown in Fig. 3, before measuring the O$_2$ dependence of the $\lambda_{Mu}$, its dependence on the concentration of each biological molecule was systematically measured with less than 1% O$_2$ in the gas phase. The increasing rate of $\lambda_{Mu}$ with increasing protein concentration was obtained as 1 $\mu$s$^{-1}$/ (g L$^{-1}$) for albumin, 1 $\mu$s$^{-1}$/ (g L$^{-1}$) for serum and 3.1 $\mu$s$^{-1}$/ (g L$^{-1}$) for Hb up to 2.0 g L$^{-1}$. Then the measurements were extended to O$_2$ dependence in 0.05 wt.% Hb aqueous solution (Fig. 4). Here, because of strong O$_2$ absorption by deoxy-Hb to change into oxy-Hb, it was expected that the Mu relaxation pattern would show a reduced response against O$_2$ increase in comparison with pure water and other protein aqueous solutions.

The result of O$_2$ concentration dependence for 0.5 g L$^{-1}$ Hb aqueous solution is shown in Fig. 4. Mu was found to take almost similar relaxation pattern with increasing O$_2$ concentration as that for pure water. Similar results were obtained for the other protein aqueous solutions. The following points were found to be evident.

1. In albumin solution, Mu relaxation takes larger increase against increasing O$_2$ concentration. The result suggests, in the presence of albumin, interaction between Mu and O$_2$ becomes more active in higher concentration.
2. In Hb solution, Mu relaxation increases with increasing O$_2$ concentration. This weakened response may be due to an influence of decreasing deoxy-Hb concentration with increasing dissolved molecular O$_2$ concentration.

4. Discussion

Before clinical application of the muon method for measuring hypoxia, it is important to conduct systematic studies on the response to O$_2$ concentrations in various biological aqueous systems. The response to O$_2$ levels in Hb aqueous solution is quite different than other proteins. Magnetic deoxy-Hb, by absorbing O$_2$, is converted to non-magnetic oxy-Hb so that an increase of O$_2$ may decrease the relaxation of Mu. This situation will be discussed separately in the followings.

4.1. Prediction of Mu response to O$_2$ in Hb aqueous solutions

For a qualitative understandings, let us assume the experimental data of Mu relaxation rate $\lambda_{Mu}$ in aqueous solution of various Hb concentration and O$_2$ concentrations be approximated way as,

$$\lambda_{Mu} = R_{Hb}(Mu) + R_{O2}(Mu).$$

The $R_{Hb}(Mu)$ is relaxation rate of Mu due to deoxy-Hb in solution, which is $k_1(\text{Hb}) \times m_{\text{deoxy-Hb}}$. There, $k_1(\text{Hb})$ (3.1 $\mu$s$^{-1}$/g L$^{-1}$) is the same as experimental data of the best fit of relaxation vs Hb concentration. Here $m_{\text{deoxy-Hb}}$ (amount of deoxy-Hb in g L$^{-1}$) is varied by an introduction of O$_2$ in aqueous solution, which is calculated using Hill’s equation [10]. It predicts deoxy-Hb fraction (deoxy-Hb/total) = $k^n/(p^n+k^n)$, where $p$ is O$_2$ partial pressure in mmHg, and $n = 2.62$ and $k = 13$ at 25°C. ($p$ in percentage is 100 × 760 %).

The $R_{O2}(Mu)$ is relaxation rate of Mu due to dissolved free molecular O$_2$ in solution. Dissolved free molecular O$_2$ in solution at $p$ in g L$^{-1}$, $c(\text{O}_2) = p \times 8.3 \times 10^{-3}/159$ (the dissolved O$_2$ in water in equilibrium with air is $8.3 \times 10^{-3}$ g L$^{-1}$, which is 159 mmHg) and the oxygen molecules bound to $m_{\text{oxy-Hb}}$ in g L$^{-1}$, $(n_{\text{Hb}}) = 4 \times m_{\text{oxy-Hb}} \times (w_{\text{O}_2}/w_{\text{Hb}})$, where $m_{\text{oxy-Hb}}$ is amount of oxy-Hb in g L$^{-1}$, and $w_{\text{Hb}}$ and $w_{\text{O}_2}$ are molecular weight of Hb (=68000 g/mol) and O$_2$ (=32 g/mol), respectively. From the best fit line of experimental data of relaxation rate of Mu vs oxygen concentration (%) in pure water, $R_{O2}(Mu)$ ($\mu$s$^{-1}$) = $\alpha_{\text{water}} \times c(\text{O}_2 \%) + \beta_{\text{water}}$, where $\alpha_{\text{water}} = 0.214(40)$ $\mu$s$^{-1}$/O$_2 \%$ and $\beta_{\text{water}} = 0.385(363)$ $\mu$s$^{-1}$, which is slightly higher than that in the more purified water [6]. Predicted result for 0.5 g L$^{-1}$ Hb is shown in Fig. 4 (blue line), where a refined experiment is under planning. The total O$_2$ in the solution (g L$^{-1}$) is $c(\text{O}_2) + n_{\text{Hb}}$.

![Figure 4. The Mu relaxation rates in higher Hb concentration aqueous solution and various O$_2$ concentrations predicted by Eq. (2)(solid lines). As seen in this figure, Mu relaxation rates become observable (below 10 $\mu$s$^{-1}$) at O$_2$ concentration lower than 10% for the Hb range below 150 g L$^{-1}$. The experimental data (open marks) at less than 1% O$_2$ concentration, zero Hb and 0.5 g L$^{-1}$ Hb are also shown as a reference.](image-url)
As discussed above, we have estimated the behaviour of Mu at different Hb concentration (upto Hb concentration in human body) as shown in Fig. 5. The observed data at less than 1% O$_2$ concentration, zero Hb and at 0.5 g L$^{-1}$ Hb concentration are also presented in the figure. As shown in Fig. 4, the relaxation rate of Mu increases with increasing Hb at any fixed O$_2$ concentration with slower increasing rate at Hb concentration higher than 1 g L$^{-1}$. It can be predicted that Mu will show undetectable fast relaxation at O$_2$ concentration lower than 5% and at higher Hb concentration around 100 g L$^{-1}$ expected for human body.

4.2. Prediction of Mu response to O$_2$ in other biological aqueous solutions
So far, other than Hb, the Mu relaxation responses to O$_2$ were done for limited cases such as albumin, serum and TBS buffer materials. There should be extended measurements before clinical application.

5. Conclusion and future perspectives
Muonium spin rotation signal was directly observed in TBS, albumin, serum and Hb aqueous solution and consistent O$_2$ concentration dependence in spin relaxation was obtained. The result is encouraging to apply the present method to a wide variety of the biological systems including human tissues. Definitely, measurements should be extended to more cases before clinical application. Human blood is composed of cells and plasma, where Hb, albumin and serum are the major components. There, the most important component should be Hb aqueous solution, which was successfully studied in the present study. As indicated in Fig. 4, the most of the Hb aqueous solution, namely, blood in human tissues, deficiency of O$_2$ concentration related to hypoxia can be monitored. Actually, in human tissues, the highest value of O$_2$ concentration is 15% at lung.

The following advantages should be emphasized for the present Mu method: (1) no need of high magnetic field like MRI, (2) no need of injection of radioactive chemicals and (3) increase of spatial resolution of the monitoring spots to 100 µm at 10 cm depth by employing an advanced muon beam [11, 12].

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