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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 O_2 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 O_2 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 ^{18}F-labelled fluoro-misonidazole (^{18}F-FMISO) tracer is widely used in PET for monitoring hypoxia. It does not directly measure molecular O_2 in the tumor, but the tracer retention affected primarily by O_2. 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 0.5×10^{-6} to 0.5×10^{-3} mol L^{-1} based on the experimental data mentioned above.

The solubility of O_2 in water at 23 °C is known as 1.29×10^{-5} mol L^{-1} kPa^{-1} [8]. Thus, under 1 atmosphere, the oxygen solubility pO_2(s.l.) at 23 °C is 1.3×10^{-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 0.4×10^{-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 3.0×10^{-1} to 3.0×10^2 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 λ_{Mu} against O_2 contents $c(O_2\%)$ 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 λ_{Mu} against ($c(O_2\%)$, 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 μ^+ to take electronic states of diamagnetic μ^+ such as μ^+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 μ^+. 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.

Biological samples adopted in the present experiment are as follows.

Albumin Bovine serum (plasma) albumin which has a molecular weight of around 66,000 and is a single polypeptide chain consisting of about 583 amino acid residues and no carbohydrates purchased from from Sigma-Aldrich Co. Ltd.

Serum Donor horse Serum purchased from TCS Biosciences Ltd. It is Sterile filtered Serum screened for Mycoplasma and Adventitous viruses.

Hemoglobin Polymerized Hb of bovine origin in a lactated Ringers solution at 13% concentration purchased as OXYGLOBIN from BIOPURE Co. Ltd. It is violet-colored and is mostly deoxy-Hb.

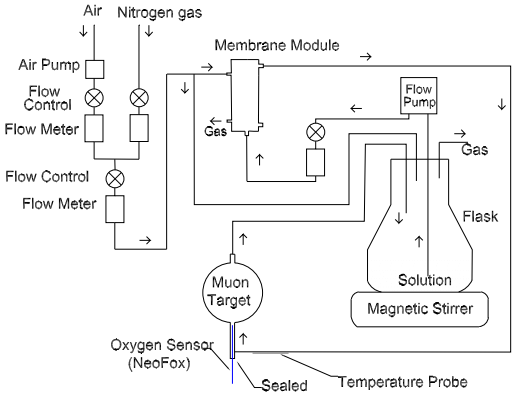

Figure 1. Diagram of biological aqueous solution production and supply to the target for the muon spin rotation experiments.

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 μ^+, 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 °C is equivalent to 1.3×10^{-4} mol L^{-1} free molecular oxygen in solution.

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

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

where N_0 is a normalization factor, B is the time-independent background and τ_μ is the muon lifetime (2.20 μs). The terms A_μ and A_{Mu} are the amplitudes of the spin precession corresponding to the polarization asymmetry for the μ^+ in diamagnetic states and in Mu, respectively. The parameter λ_{Mu} is the muonium relaxation rate while the relaxation rate of μ^+ in diamagnetic species is assumed to be negligible, ω_μ and ω_{Mu} are the muon and Mu precession frequencies, ϕ_μ and ϕ_{Mu} are the respective initial phases of their precessions. Under transverse field of H(G), the spins of μ^+ and Mu take precession with angular velocity of ω_μ(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 λ_{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 λ_{Mu} with increasing protein concentration was obtained as 1 $\mu s^{-1}/(\text{g L}^{-1})$ for albumin, 1 $\mu s^{-1}/(\text{g L}^{-1})$ for serum and 3.1 $\mu s^{-1}/(\text{g L}^{-1})$ 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.

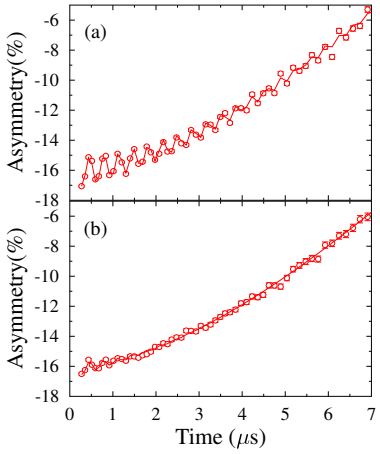

Figure 2. Muon spin rotation time spectrum in water with O_2 concentration (a) below 1% and (b) 7.5%, at room temperature under 2.1 G transverse field. In most of the measurements on other biological aqueous solutions, almost similar O_2 dependence was obtained.

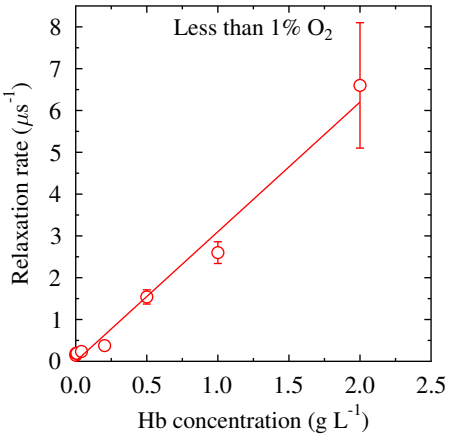

Figure 3. Relaxation rate of Mu against Hb concentration in water at less than 1% O_2 concentration.

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 is weakened. 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 λ_{Mu} in aqueous solution of various Hb concentration and O_2 concentrations be approximated way as,

$$\lambda_{Mu} = R_{Hb}(Mu) + R_{O_2}(Mu). \quad (2)$$

The R_{Hb}(Mu) is relaxation rate of Mu due to deoxy-Hb in solution, which is k_1(Hb)$\times m_{deoxy-Hb}$.There, k_1(Hb) (3.1 μs^{-1}/(g L^{-1})) is the same as experimental data of the best fit of relaxation vs Hb concentration. Here $m_{deoxy-Hb}$ (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 $100 \times p/760$ %).

The R_{O_2}(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}, $c(O_2) = p \times 8.3 \times 10^{-3}/159$ (the dissolved O_2 in water in equilibrium with air is 8.3×10^{-3} g L^{-1}, which is 159 mmHg) and the oxygen molecules bound to m_{oxy-Hb} in g L^{-1}, $(n_{Hb}) = 4\times\ m_{oxy-Hb} \times (w_{O_2}/w_{Hb})$, where m_{oxy-Hb} is amount of oxy-Hb in g L^{-1}, and w_{Hb} and w_{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_{O_2}(Mu) (μs^{-1}) = $\alpha_{water} \times c(O_2\%) + \beta_{water}$, where α_{water} = 0.214(40) $\mu s^{-1}/(O_2\%)$ and β_{water} = 0.385(363) μs^{-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 $c(O_2) + n_{Hb}$.

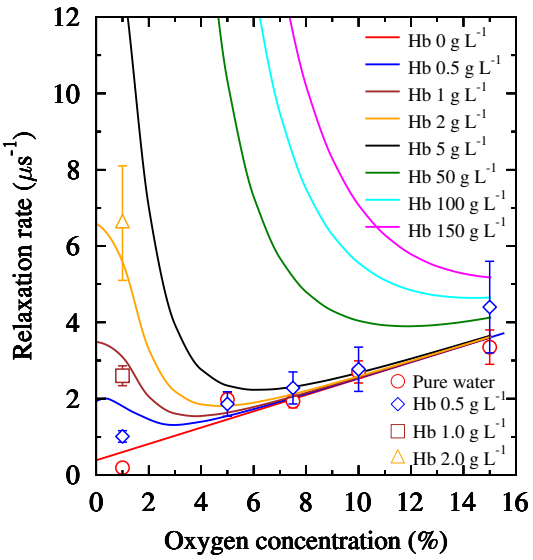

Figure 4. The Mu relaxation rates in higher Hb concentration aqueous solution and various O_2 concentrations predicted by Eq. (2)(solid lines). As seen in this figure, Mu relaxation rates become observable (below 10 μs^{-1}) at O_2 concentration lower than 10% for the Hb range below 150 g L^{-1}. The 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.

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 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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References
[1] Mancini D M, Wilson J R, Bolinger L, Li H, Kendrick K, Chance B and Leigh J S 1994 *Circulation* **90** 500
[2] Raleigh J A, Dewhirst M W and Thrall D E 1996 *Semin. Radiat. Oncol.* **6** 37
[3] Kavanagh M C, Tsang V, Chow S, Koch C, Hedley D, Minkin S and Hill R P 1999 *Int. J. Radiat. Oncol. Biol. Phys.* **44** 1137
[4] Tatum J L *et al.* 2006 *Int. J. Radiat. Biol.* **82** 699
[5] Jean Y C, Fleming D G, Ng B W and Walker D C 1979 *Chem. Phys. Lett.* **66** 187
[6] Nagamine K, Nishiyama R, Imazato J, Nakayama H, Yoshida M, Sakai Y, Sato H and Tominaga T 1982 *Chem. Phys. Lett.* **87** 186
[7] Roduner E, Tregenna-Piggott P L W, Dilger H, Ehrensberger K and Senba M 1995 *J. Chem. Soc. Faraday Trans.* **91** 1935
[8] Dean J A 1999 *Lange's Handbook of Chemistry* (New York: McGraw-Hill) 15th Editon pp. 5.6
[9] Pauling L and Coryell C D 1936 *Proc. Nat. Acad. Sci.* **22** 210
[10] Voet D and Voet J 2004 *Biochemistry* (New York: John Wiley and Sons)
[11] Miyadera H *et al.* 2007 *Proceedings of PAC07* 3032
[12] Nagamine K 2014 *JPS Conf. Proc.* **2** 010001