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New life science studies with muons and radioactive nuclei

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Abstract. Electron-transfer phenomena in biological macromolecules are among the most important processes in physics. Since there are no "radioactive electrons", a limited number of microscopic studies exist, and thus most knowledge is based upon macroscopic studies. In order to overcome this situation, a muon-labelled electrons method was recently developed and successfully applied to directly exploring microscopic electron-transfer phenomena in representative proteins, such as cytochrome-c, cytochrome-c oxidase and myoglobin as well as DNA. The principle and some details of each experiment are reviewed. A possible extension to the labelled-electron method with radioactive nuclei is discussed.

PACS. 76.75.+i Muon spin rotation and relaxation – 87.15.-v Biomolecules: structure and physical properties – 87.66.Uv Magnetic resonance

1 Introduction

The electron-transfer process in macromolecules, such as proteins, is an important part of many biological phenomena, such as the storage and consumption of energy and photosynthesis. A number of experimental investigations have been carried out to explore the electron-transfer phenomena in proteins and related chemical compounds. However, almost all of the existing information on electron transfer has been obtained by essentially macroscopic methods. In order to understand the details of electron transport, it is thus very important to use methods that provide information at a more microscopic level.

Recently, by extending the muon spin rotation/relaxation/resonance (μSR) method, we have successfully developed a method to directly observe microscopic aspects of electron transfer. The principle of μSR is based upon the particle-physics law of weak interactions of polarized muon production via pion-decay as well as asymmetric e^+/e^- emission from a polarized muon. As shown in fig. 1, the spin of the μ^+ (positive muon), when it is born by the decay of a π^+ (positive pion), is completely polarized along the direction of its motion; once the μ^+ is obtained as a beam, it is polarized along the beam direction. During the slowing-down of the μ^+ inside the host material, the spin polarization is completely maintained. After thermalization and occupation at a specific microscopic location, the μ^+ decays into an e^+ and two neutrinos (as shown in fig. 1), and the e^+ takes a spatial distribution preferentially along the μ^+ spin. There, because the e^+ energy goes up to 50 MeV, the direction of the μ^+ spin can be detected time by time by measuring the high-energy e^+ using detectors placed outside of the target material to be investigated.

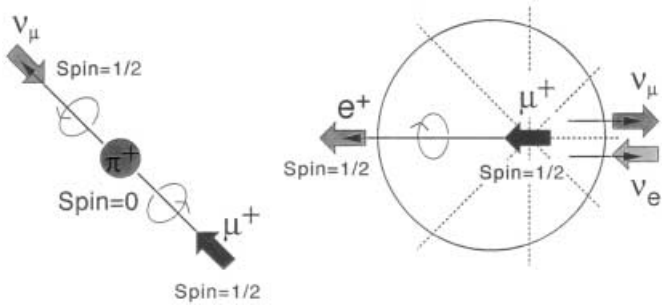

Fig. 1. Muon spin polarization originating from positive-pion decay (left), and the spacial distribution of the positron from the decay of a polarized muon (right).

A schematic view of the experimental arrangement is shown in fig. 2, where a sample with a typical size of around 1 cm^2 area and a few 100 mg/cm^2 thickness is placed along the μ^+ beam direction. The sample is surrounded by an array of e^+ detectors; as can be seen in fig. 2, in the case of μSR experiments with an intense pulsed-muon beam, the e^+ detector is highly segmented in order to eliminate any counting loss. The fast electronics of the time digitizer as well as an on-line data-taking system are connected to measure the time dependence of the anisotropic e^+ intensity with reference to the time of the μ^+ arrival. Thus, one can take the time dependence of the intensity ratio between the forward and backward counters to measure the e^+ asymmetry to monitor the time evolution of the μ^+ spin polarization.

With the help of theoretical developments initiated by the late Professor Kubo, the observed time evolution of the μ^+ spin direction, seen in the time spectrum of the

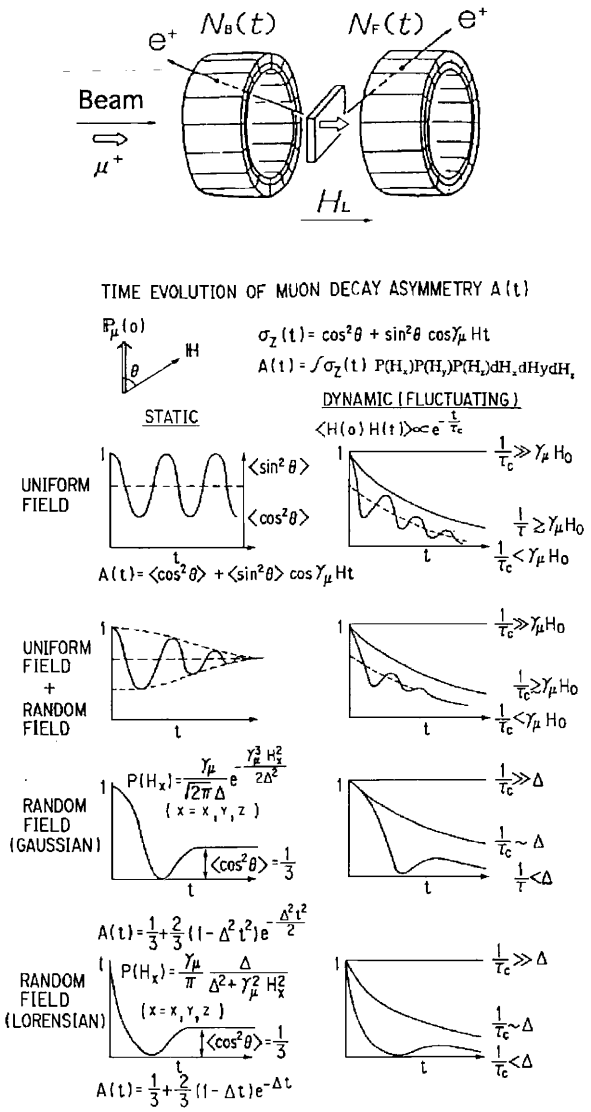

Fig. 2. Typical layout of the μSR experiment with pulsed muons (above), and the μSR time spectrum expected from the magnitude, distribution and fluctuation of the local fields at the muon (below).

asymmetry of anisotropic e^+ decay, can have a one-to-one correspondence to the static as well as dynamic nature of the microscopic magnetic field seen by the μ^+ (see fig. 2).

Thus, the μSR method can be considered to be a sensitive magnetic compass to probe the microscopic magnetic properties of condensed matter [1]. The excellent features of the μSR method, in comparison with those of other microscopic probes, like neutron scattering, synchrotron radiation and NMR, can be summarized as follows: i) because spin polarization is provided by the particle-physics law, as mentioned above, the microscopic magnetic properties can be studied under zero external field, which is a significant advantage for studies of superconductors; ii) sensitive measurements of microscopic fields with a capability of the high-efficiency detection of radioactive products can be realized by employing the high flux of

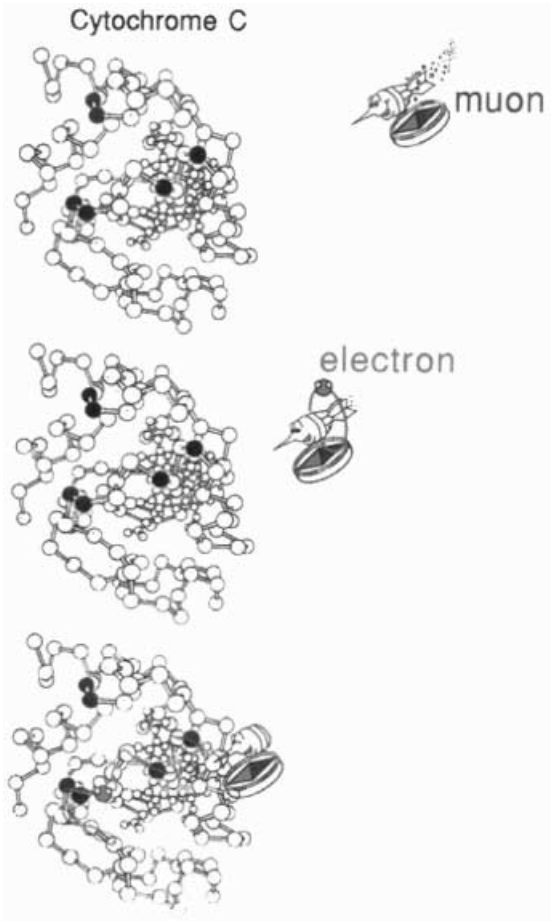

Fig. 3. Schematic diagram representing the role of the μ^+ probe for electron transfer in a macromolecule: injection of a high-energy μ^+ (top); electron pick-up and muonium (μ^+e^-) formation during slowing-down (middle); thermalization of the muonium followed by the electron release at the chemical bonding and sensitive detection of the characteristic electron motion in the macromolecule via magnetic interaction (bottom).

a μ^+ beam, where 1 M e^+ events/min can easily be obtained; iii) with the help of the large μ^+ magnetic moment (3.2 times that of the proton) as well as the "short" lifetime of the μ^+ (2.2 μs), the μSR method is sensitive to a very weak (down to less than one gauss) and randomly oriented microscopic magnetic field; iv) again, with the large magnetic moment as well as the available time window for a μSR measurement, the μSR is sensitive to the dynamics of the surrounding electronic spin, whose characteristic correlation time (τ_c) is $10^{-11}\,\mathrm{s} < \tau_c < 10^{-5}$ s, which is somewhat slower than that detected by neutron scattering ($\tau_c < 10^{-12}$ s), since the in-flight neutron can only sense a field dynamics corresponding to the time of passage; v) finally, time domain information of the spin motion of the μ^+ probe can be directly obtained, in contrast to NMR or ESR, where frequency domain information is the only accessible information, enabling the μSR to act as a sensitive probe to spin dynamics.

In order to obtain microscopic information about electron transfer in a biological macromolecule, the muon

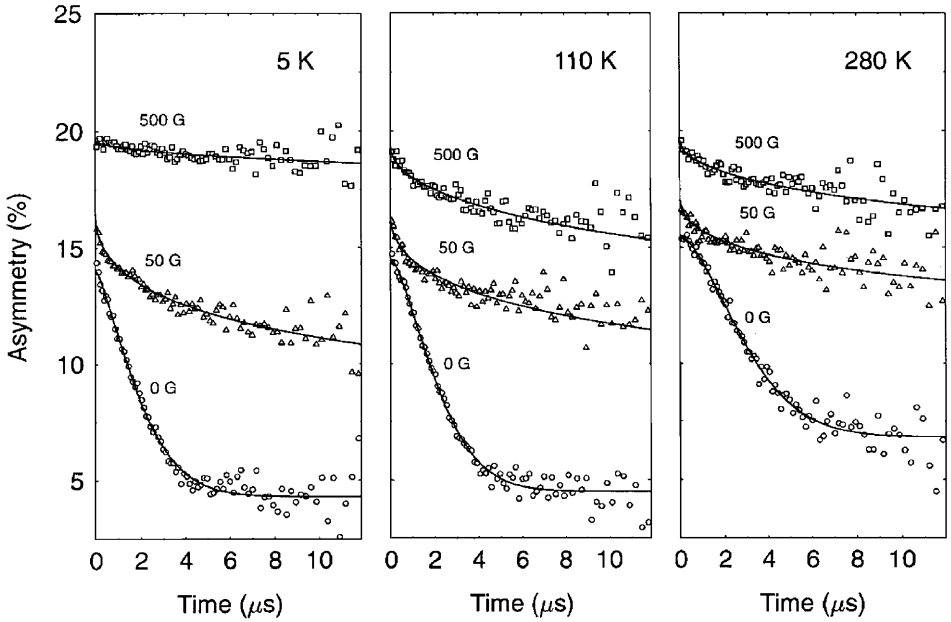

Fig. 4. Typical μ^+ spin-relaxation time spectra in cytochorome-c at 5 K, 110 K and 280 K under external longitudinal fields of 0 G, 50 G and 500 G. For finite fields the curves show fits using the *R-K* function.

spin-relaxation (μSR) method offers great potential (see, fig. 3). During the slowing-down process, the injected μ^+ picks up one electron to form a neutral atomic state, called a muonium. This muonium is then thermalized, followed by chemical bonding to a reactive site on the molecule. Then, depending upon the nature of the molecule, the electron brought in by the μ^+ can take on several characteristic behaviours, including localization to form a radical state and/or a linear motion along the molecular chain. These behaviours, by setting the time-origin of electron movement, can be detected most sensitively by measuring the spin-relaxation process of the μ^+ using the μSR method, which occurs through a magnetic interaction between the μ^+ and the moving electron produced by the μ^+, itself. In other words, in place of "radioactive electrons", by introducing "electron" and an "electron observer" at the same time, the tracer of the "electron" can be made as an alternative manner: the labelled-electron method.

This idea of sensitive detection of the electron behaviour in macromolecules using muons has been successfully applied in a series of studies of electron transport in conducting polymers [2,3]. A soliton-like motion has been studied for a μ^+-produced electron in trans-polyacetylene, which contrasts with the localization seen in cis-polyacetylene following the formation of a radical state [2]. Similarly, polaron-type electron transport phenomena in polyaniline have also been studied [3].

In μ^+ spin-relaxation measurements under an applied longitudinal magnetic field, due to the nature of the dipole-dipole magnetic interaction between the moving electrons and the stationary muons, the characteristic dimensionality of the electron motion can be studied based on the dependence of the muon spin-relaxation rate (λ_μ) upon an externally applied magnetic field ($B_{\rm ext}$); for one-dimensional electron motion, $\lambda_\mu \propto (B_{\rm ext})^{-1/2}$, for two-dimensional electron motion, $\lambda_\mu \propto (\alpha - \beta \log B_{\rm ext})$, and for three-dimensional electron motion, λ_μ does not usually have a significant $B_{\rm ext}$-dependence [4].

Progress has been made in the theoretical understanding of this paramagnetic relaxation process by Risch and Kehr, who considered a stochastic treatment of the random-walk process of a spin which is rapidly diffusing along a topologically one-dimensional chain [5]. An error-function type longitudinal relaxation function (hereafter called the *R-K* function), $G(t) = \exp(\Gamma t)\mathrm{erfc}(\Gamma t)^{1/2}$, was proposed for $\lambda t_{\rm max} \gg 1$, where λ is the electron spin-flip rate, $t_{\rm max}$ the experimental time scale and Γ a relaxation parameter. In this theoretical treatment, Γ is proportional to $1/B_{\rm ext}$.

2 New life science studies with muons

2.1 Probing electron transfer in cytochrome-c and myoglobin

Among various types of proteins, cytochrome-c has attracted much attention, because it plays an essential role in the respiratory electron-transport chain in mitochondria, occupying a position next to the final process of the cycle, in which it transfers electrons to the surrounding oxidase complex. On the other hand, myoglobin is known to be important in oxgen transport with a basic molecular structure similar to that of the electron-transfer protein.

Experiments on μ^+ relaxation in cytochrome-c and myoglobin have been conducted using an intense pulsed beam (70 ns pulses at 50Hz repetition rate) of 4 MeV μ^+ at the RIKEN-RAL Muon Facility. The essential part is summarized in the following, and further details should

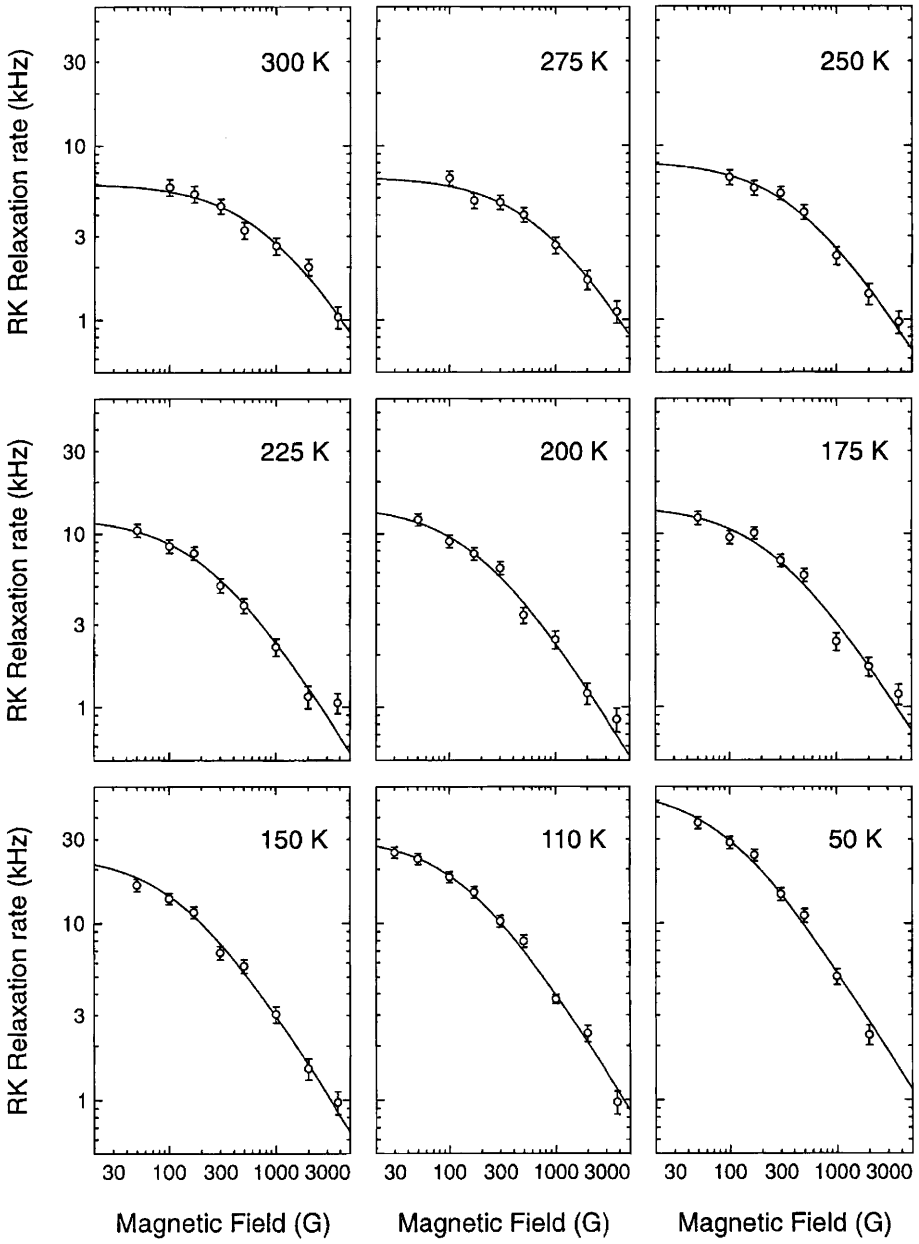

Fig. 5. *R-K* relaxation parameter, Γ, *versus* the external longitudinal magnetic field for the μ^+ in cytochrome-c at typical temperatures. The B^{-1}-dependent part can be seen to become significant in the higher field region, and the critical field (cutoff field) of the onset of the B^{-1}-dependence can be seen to have a clear temperature dependence.

be found in a published report [6]. The cytochrome-c used here is of the Fe^{+++} type in a polycrystalline powder form, extracted from horse heart (Wako-Chemical product), while myoglobin was extracted from horse muscle (Sigma Co. Ltd.). As references, cytochrome-c with Fe^{++} prepared by Drs M. Ataka and T. Kubota and lysozyme extracted from chicken egg (Sigma Co. Ltd.) were used. The present μ^+SR measurements were carried out for a range of temperatures between 5 K and 300 K and for a number of longitudinal magnetic fields in the range of 0 to 0.4 T. All measurements were conducted on an as-received powder sample.

At each of the measurement temperatures, the μ^+ relaxation function was found to depend on an external field in the cases of cytochrome-c with Fe^{+++} and myoglobin. Some typical relaxation curves are shown in fig. 4; it can be seen that the spin relaxation becomes suppressed at higher external fields, $B_{\rm ext}$, while the initial asymmetry at $t = 0$, $a_\mu(0)$, increases with $B_{\rm ext}$. The observed relaxation functions, $G(t)$, were fitted with the *R-K* function [5] and the longitudinal relaxation parameter, Γ, obtained at various temperatures is seen to decrease monotonically with increasing $B_{\rm ext}$ (fig. 5). Upon a closer inspection of the $B_{\rm ext}$-dependence of Γ, there are two components: 1) a region

of weak field dependence (lower field) and 2) a $(B_{\rm ext})^{-1}$-dependent region (higher field). The latter region exhibits the characteristic μ^+ spin-relaxation behaviour due to the linear motion of a paramagnetic electron. As can be seen in fig. 5, the critical field (hereafter we refer to this as the cutoff field) where the second region takes over from the first one, has a significant temperature dependence; the cutoff field is seen to reduce with decreasing temperature. Similar measurements performed for both lysozyme and cytochrome-c with Fe (II) did not show any similar region where Γ follows a $(B_{\rm ext})^{-1}$-dependence.

All of these results lead to the following microscopic picture of the electron-transfer process for a muon-injected electron in cytochrome-c and myoglobin:

1) Topologically linear motion exists along the chain of both cytochrome-c and myoglobin.
2) The intersite diffusion rate along chain $D_{||}$, which can be obtained from the measured Γ and the hyperfine coupling constant (500 MHz) estimated from the recovery curve of the initial asymmetry, takes on a value on the order of 10^{12} rad/s, and is almost temperature independent (fig. 6). In the case of cytochrome-c, this value is consistent with those obtained using optical methods in ruthenated proteins for short electron-transfer distances in the region of 5 Å [7].
3) The cutoff process represents a departure toward a longer time scale from the topologically one-dimensional diffusion, which occurs in a shorter time scale. A naive picture based upon previous μ^+SR studies on high-molecular-weight conducting polymers would suggest an increase in the effective dimensionality of the diffusion at temperatures higher than 200 K due to an increased interchain diffusion rate, $D_\perp$. Assuming that the crossover occurs as the interchain diffusion rate becomes comparable with the electronic Larmor frequency, one can obtain the interchain diffusion rate, $D_\perp$. Depending upon the temperature region, the obtained $D_\perp$ in cytochrome-c is dominated by two different processes, as can be seen in the temperature dependence (fig. 6): one with a characteristic activation energy of 150 meV, seen above 200 K, and the other with an activation energy of less than 2 meV, seen below 200 K. The characteristic change at 200 K of $D_\perp$ seems to be related to the well-known structural change of some proteins, which has been suggested to be a glass-like transition [8]. In the context of a protein such as cytochrome-c, with coils and folds in its structure, the "interchain" diffusion might be interpreted as "interloop" jumps, which could be strongly activated by the increased thermal displacement of the polymer occurring above the glass transition. On the other hand, $D_\perp$ in myoglobin shows only one component, reflecting the different protein dynamics of this molecule for interchain electron transfer.

The most important unknown pieces of information are the distribution of the locations of the μ^+ bonding-sites and the corresponding electronic structure around the μ^+ at the site from where the electron starts its linear motion. For this purpose, muon-spin RF resonance has been

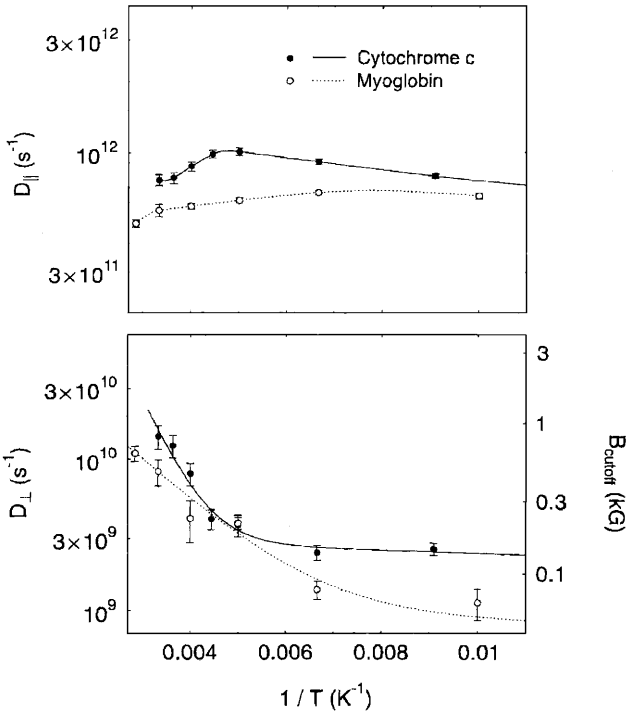

Fig. 6. Upper plot showing the temperature dependence of the parallel diffusion rate of an electron in cytochrome-c and myoglobin derived from the B^{-1}-dependent part of the relaxation curve. The lower plot shows the perpendicular diffusion rate, derived from the cutoff field determined in fig. 5, plotted against the inverse temperature.

conducted on the μ^+ in both cytochrome-c and myoglobin. The results gave about a 10 ppm paramagnetic shift with a characteristic temperature dependence; more precise measurements will provide information on the distance of the μ^+ location from the heme Fe.

Although, as mentioned above, there are still some unknown factors at this stage concerning the detailed nature of the μ^+ probe state, it is clear that the electron-transfer process through a microscopic section of either cytochrome-c or myoglobin was directly detected in the present experiment. The present μSR method should be contrasted with experiments using photo-excited electrons, where the electron transfer is measured through a path connecting two specific sites. The μ^+SR method, of which the high efficiency of the measurements should be emphasized, has the ability to extend these studies to any kinds of proteins in various chemical and biological environments: *e.g.* in solution with different values of pH, etc.

2.2 Probing electron transfer in cytochrome-c oxidase

In recent experimental studies conducted at the RIKEN-RAL muon facility, work was extended to explore electron transfer in the cytochrome-c oxidase, which is known to be a terminal protein situated at the final part of the mitochondria aspiration chain, in collaboration with Drs.

S. Yoshikawa, and K. Shinzawa-Itoh of Himeji Institute of Technology and T. Tsukihara of Institute for Protein Research, Osaka University. Recently, structure studies have been carried out using high-resolution X-ray studies [9]. Due to a difficulty in sample preparation, the actual sample was composed of the Bovine heart cytochrome-c oxidase (50%) and the surfactant material Deyl-β-D-maltoside and buffer chemicals of NaH_2PO_4 (altogether 50%).

The results are shown in figs. 7a and 7b in terms of the external-field dependence of the relaxation parameter (Γ) obtained by a fit to the data with the Risch-Kehr function representing a relaxation function due to a lineraly moving electron. The following conclusions were readily obtained:

1) At room temperature, electron transfer along the chain in cytochrome-c oxidase is very much suppressed compared to that in cytochrome-c and mioglobin.
2) By reducing the temperature, the electron transfer along the chain becomes evident, particularly below 150 K.

The results may contain contributions of the signals from either surfactant or the buffer. Since there exist several heme centers, there might be corresponding signal components. Extended studies are now in progress.

2.3 Probing electron transfer in DNA

Electron-transfer phenomena in DNA are known to be important not only concerning damage and repair mechanisms, but also because of possible applications to new bio-devices. A recent experimental finding [10] of a possible electron transfer between G bases has accelerated both experimental and theoretical studies. Recently, at RIKEN-RAL Muon Facility, the Yamanashi U.-RIKEN-KEK-Oxford-Tokyo Kasei Gakuen U.-Juelich collaboration, has successfully conducted a μSR experiment on oriented DNA in both A and B form DNA, and observed electron transfer in DNA, somewhat consistent with the picture of an electron hopping through base pairs [11].

3 Possible new life science studies with radioactive nuclei

Let us consider the possibility of a labelled electron method with radioactive nuclei. Anticipating the use of the in-beam Perturbed Angular Correlation (PAC) method, we can propose the following possibilities, where electron production as well as the existence of polarized radioactive nuclei are the key-components to be prepared:

i) The use of β-decay electrons and residual polarized nuclei. In this case, because most electrons may have energies ranging from keV to MeV, it is difficult to expect these electrons to take the role of biological electrons.

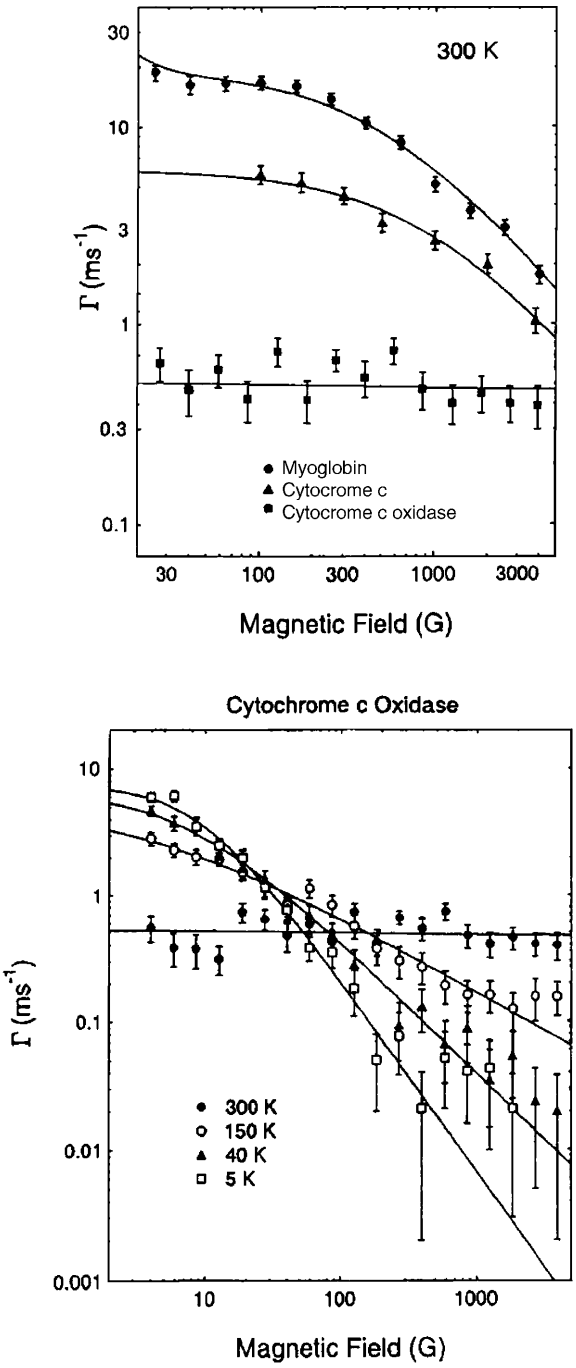

Fig. 7. Observed muon spin relaxation parameter under various external magnetic fields on three typical proteins at room temperature (top). Same for cytochrome-c oxidase at various temperatures (bottom).

ii) Introduce polarized nuclei by using electrons brought by the nuclei during a slowing-down process. This idea exactly follows the method of muon-labelled electrons. During the slowing-down of the nuclei, a loss and capture of electrons in the ionization process is not so simple, like in the case of positive muons with $Z = 1$. Competing processes may disturb a simple picture of

the formation and preservation of the paramagnetic atomic state before thermalization. An attempt should be made to apply highly polarized nuclei, like ^{8}Li or ^{12}B, etc., which are easily produced in low-energy nuclear reactions, to the labelled-electron method.

iii) Use of electron(s) filling the hole(s) in the K-capture process. Immediately after hole-filling, some Auger electron(s) are produced. They might follow the process of biological electron transfer. By succeedingly detecting γ-rays though their polarization, if necessary, one can learn about the interaction between the moving Auger electrons and the residual polarized nuclei. The required conditions of the labelled electron method might be satisfied.

4 Conclusion

By using positive muons, the labelled-electron method was proved to be a promising probe to explore microscopic aspects of electron-transfer phenomena in biological molecules. For the eventual future, we can expect the role of positive muons as a monitor for the overall features of electron transfer, while that of radioactive nuclei as a monitor for site-selected features.

Biological electron transfer has a diffusion time constant ($\tau_{\rm c}$) from ps/site to ns/site. By considering the value of the average fluctuating hyperfine fields from the moving electrons to the radioactive probe (ω) being 10^{7}–10^{10} Hz, the relaxation time of the probe estimated by the $(\omega^{2}\tau_{\rm c})^{-1}$ relation becomes in the range from 10^{-9} s to 10^{-2} s, nicely matching the lifetime of either the muon or radioactive nuclei. Thus, life science studies with these radioactive probes should be highly encouraged.

The author acknowledges helpful contributions by Drs F.L. Pratt, I. Watanabe and E. Torikai to the pioneering μSR studies on life science subjects. The muon experiments described here were mainly conducted at the RIKEN-RAL muon facility. The author acknowledges related persons of both RIKEN and RAL for their kind support and encouragement.

References

1. For recent activities, see, *e.g., Proceedings of the 7th International Conference on μSR, Nikko, April 1996*, edited by K. Nagamine, K. Nishiyama, R.M. Macrae, R. Kadono, Hyperfine Interact. **104-106** (1997); *Proceedings of the 8th International Conference on μSR, Les Diablerets, August 1999*, edited by E. Roduner, A. Schenck, G. Solt, Physica B **289-290** (2000).
2. K. Nagamine, K. Ishida, H. Shirakawa, *et al.*, Phys. Rev. Lett. **53**, 1763 (1984); K. Ishida K. Nagamine, H. Shirakawa, *et al.*, Phys. Rev. Lett. **55**, 2209 (1985).
3. F.L. Pratt *et al.*, Phys. Rev. Lett. **79**, 2855 (1997).
4. M.A. Butler, L.R. Walker, Z.G. Soos. J. Chem. Phys. **64**, 3592 (1976).
5. R. Risch, K.W. Kehr. Phys. Rev. B **46**, 5246 (1992).
6. K. Nagamine, F.L. Pratt *et al.*, Physica B **289-290**, 631 (2000).
7. C.C. Moser, J.M. Keske, K. Warncke, R.S. Farid, P.L. Dutton, Nature **355**, 796 (1992).
8. *E.g.*, F. Parak, E.N. Frolov, R.L. Mössbauer, V.I. Godanskii, J. Mol. Biol. **145**, 825 (1981).
9. T. Tsukihara *et al.*, Science **269**, 1069 (1995).
10. E. Meggers *et al.*, J. Am. Chem. Soc., **120**, 12950 (1998).
11. E. Torikai, K. Nagamine, H. Urabe *et al.*, to be published in Hyperfine Interact. (2002).