Intramolecular electron transfer in cytochrome c observed by the muon spin relaxation method

Kanetada Nagamine,[*1,*2] Francis L. Pratt,[*1,*3] Seiko Ohira,[*1,*2,*4] Isao Watanabe,[*1]
Katsuhiko Ishida,[*1] Satoshi N. Nakamura,[*1] and Teiichiro Matsuzaki[*1]
[*1] *Muon Science Laboratory, RIKEN*
[*2] *Meson Science Laboratory, Institute of Materials Structure Science, High Energy Accelerator Research Organization (KEK-MSL)*
[*3] *Department of Physics, The Clarendon Laboratory, University of Oxford, UK*
[*4] *Department of Physics, School of Science, University of Tokyo*

Rapid electron transfer motion in cytochrome c was studied microscopically for the first time using muon spin relaxation. The spin relaxation rate of an injected positive muon is caused by the magnetic interaction between the spin of the stationary muon and the spin of the moving electron produced by the injected muon itself. Temperature dependent measurements from 5 K to room temperature indicate that the fundamental intersite difusion rate for the topologically one-dimensional electron motion along the polypeptide chain is only weakly dependent on temperature. Evidence for an increase in higher dimensional motion is seen around 200 K, apparently reflecting a structural change in cytochrome c and opening up thermally activated tunneling paths which short-circuit the diffusive motion along the main chain.

Electron transfer processes in macromolecules such as proteins are an important part of biological phenomena, such as the storage and consummation of energy, photo-synthesis and brain function. A number of experimental investigations have been carried out to explore the electron transfer phenomena in proteins and related chemical compounds. One of the key methods to date for measuring a long-distance electron transfer is to measure the electron-transfer kinetics from a reduced heme (Fe^{2+}) to an oxidized surface-bound Ru complex (Ru^{3+}) using a flash-quench procedure, thus concentrating on the specific path of the electron transfer.[1] However, almost all the existing information on the electron transfer have been obtained by essentially macroscopic methods, which measure the evolution of the complete electron transfer from donor to acceptor. Long range electron transfer however involves complex paths involving many tunneling segments, each of which will influence the total transfer rate. In order to understand the details of the electron transport it is thus very important to use methods that provide information at a more microscopic level.

Among various types of proteins, cytochrome c attracted much attention, since 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.[2,3] The three-dimensional molecular structure of cytochrome c is well known, since determination by X-ray structural analysis in the sixties.[4]

The use of muon spin relaxation in electron-transfer studies

In order to obtain microscopic information on the electron transfer in the macro-molecule, the muon spin relaxation (μSR) method offers great potential. Polarized positive muons (μ^+), available as a particle beam in the MeV energy region from a beam channel installed at a high energy proton accelerator facility, can be injected into a biological substance. During the slowing-down process, the injected μ^+ picks up one electron to form a neutral atomic state called muonium, which is in many ways analogous to a hydrogen atom. The 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 can be detected most sensitively by measuring the spin relaxation process of the μ^+ using the μSR method. The muon spin relaxation occurs through a magnetic interaction between the μ^+ and the moving electron produced by the μ^+ itself. The μ^+ spin relaxation is observed by detecting the time-dependent change of the spatially anisotropic distribution of positrons emitted preferentially along the μ^+ spin direction after the radioactive decay of polarized μ^+ (lifetime of 2.2 μs). The initial μ^+ polarization is naturally obtained in the production process of pion-decay, following the laws of particle-physics. Here, the μ^+ takes on a double role, both as an injector of an electron and also as a sensitive observer of the electron's behaviour.

This idea of the sensitive detection of the electron behaviour in macromolecules using muons has been applied successfully in studies of electron transport in conducting polymers.[5-7] A soliton-like motion has been studied for the μ^+-produced electron in trans-polyacetylene, which contrasts with the localization seen in cis-polyacetylene following the formation of a radical state.[5,6] Similarly, polaron-type electron transport phenomena in polyaniline have also been studied.[7]

The most significant observables in these μSR studies can be summarized as follows. In longitudinal relaxation measurements, due to the nature of the interaction between the moving electrons and the stationary muons, the characteristic dimensionality of the electron motion can be studied by varying the externally applied field ($B_{\rm ext}$) and following the dependence of the muon spin relaxation rate (λ_μ) ; for one-dimensional electron motion $\lambda_\mu \propto (B_{\rm ext})^{-1/2}$, for two-dimensional electron motion $\lambda_\mu \propto (\alpha - \beta \log B_{\rm ext})$, where α and β are constants, and for three-dimensional electron motion λ_μ does not have significant $B_{\rm ext}$ dependence.[8)]

Progress has been made in the theoretical understanding of this paramagnetic relaxation process by Risch and Kehr who considered the direct stochastic treatment of the random-walk process of a spin which is rapidly diffusing along a topologically one-dimensional chain.[9)] An error-function type longitudinal relaxation function (hereafter called 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 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 $1/B_{\rm ext}$ in the case of topologically one-dimensional electron motion. In a recent experiment,[7)] the usefulness of the R-K function has been confirmed experimentally for the polaronic motion of conducting electrons in polyaniline.

As a natural extension of this type of application of the μSR method, possible studies of electron transfer in protein was proposed in 1987 by one of the present authors.[10)] The only previous report of μSR on biological molecules was an early study back in 1979, demonstrating muonium reaction with various nucleotides and with DNA.[11)] However, no μSR studies on electron motion in proteins have been reported up to now.

μSR experiment on cytochrome c

Experiments on the μ^+ relaxation in cytochrome c have been conducted by using an intense pulsed beam (70 ns pulses at 50Hz repetition rate) of 4 MeV μ^+ at the RIKEN-RAL Muon Facility[12)] at Rutherford Appleton Laboratory, UK. By using a μSR spectrometer which has a large solid-angle, highly-segmented detector arrays, a precise measurement of the spin relaxation function of the μ^+ for times up to 30 μs can be made in a relatively short data acquisition time (1 M events/min). As established in the earlier work on a range of muon science experiments,[12-14)] the use of pulsed muons is essential for measuring long relaxation times that appear in the present type of spin relaxation studies.

The cytochrome c used here is Fe(3+) type in a polycrystalline powder form extracted from horse heart (Wako-Chemical product). The present μSR 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, applied along the μ^+ initial spin direction. All measurements were conducted on the powder sample as received.

At each of the measurement temperatures, the μ^+ relaxation function, which corresponds to the time-dependent change of the μ^+ polarization, was found to have an external field dependence. Some typical relaxation curves are shown in Fig. 1; 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 against $B_{\rm ext}$ (note that the time dependent change of the spin polarization is described by $a_\mu(0)G(t)$). The observed relaxation functions $G(t)$ were fitted by the R-K function[9)] and the longitudinal relaxation parameter Γ obtained at various temperatures is seen to decrease monotonically with increasing $B_{\rm ext}$ (Fig. 2). On closer

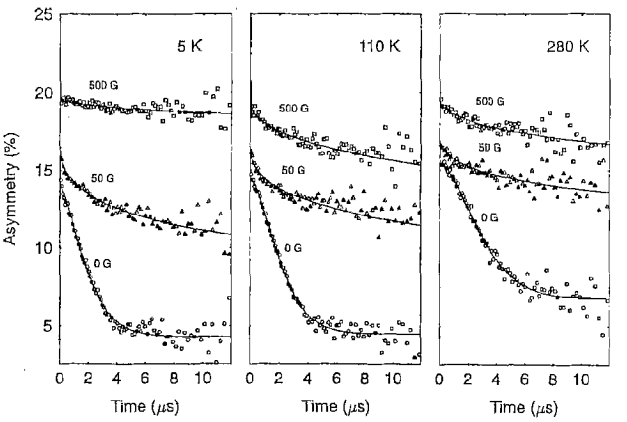

Fig. 1. Typical μ^+ spin relaxation time spectra in cytochrome c at 5, 110 and 280 K under external longitudinal fields of 0, 50 and 500 G. For finite fields the curves show best fits using the R-K function.

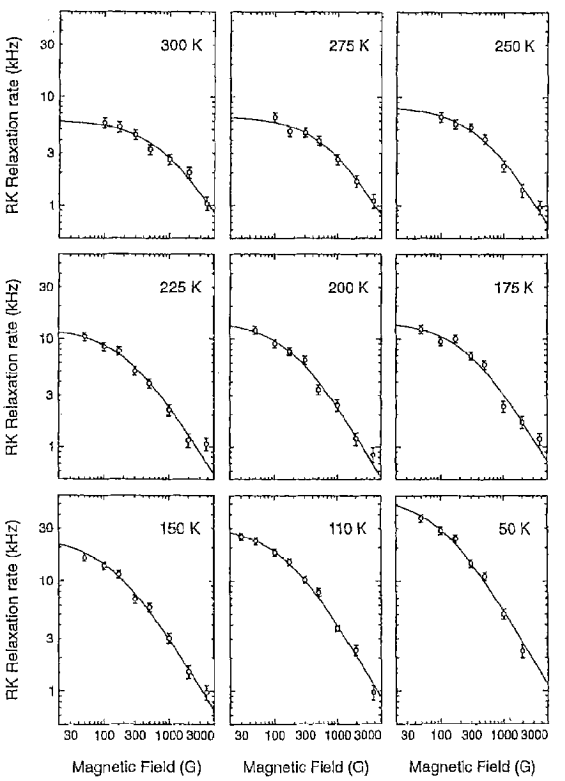

Fig. 2. The RK relaxation parameter Γ versus external longitudinal magnetic field for the μ^+ in cytochrome c at various temperatures. The B^{-1} dependent part can be seen to become significant at higher field region and the critical field (cutoff field) of the onset of the B^{-1} dependence can be seen to have a clear temperature dependence.

inspection of the B_{ext} dependence of Γ, there seems to be two components, (1) a region of weak field dependence (lower field) and (2) a $(B_{ext})^{-1}$ dependent region (higher field). The latter region exhibits the characteristic μ^+ spin relaxation behaviour due to linear motion of a paramagnetic electron. As seen in Fig. 2, the critical field (hereafter we refer to this as the cutoff field) where the second region becomes significant over the first region has a significant temperature dependence; the cutoff field is seen to reduce with decreasing temperature. An Arrhenius plot of the cutoff field against inverse temperature is shown in Fig. 3. It can be seen that the temperature dependence of the cutoff field can be presented by the sum of two activated components of the form $\exp(-E_a/kT)$, where E_a is an activation energy; one with an activation energy of 150 meV (dominant over 200 K) and the other with an activation energy of less than 2 meV (dominant below 200 K).

Over the entire temperature range the initial amplitude $a_\mu(0)$ recovers with increasing field. It shows a first recovery at lower field (up to 300 G) and then it shows a second recovery at higher field (above 300 G). This can be taken as evidence for the existence of two different classes of muon site with differing muon-electron hyperfine coupling constants.

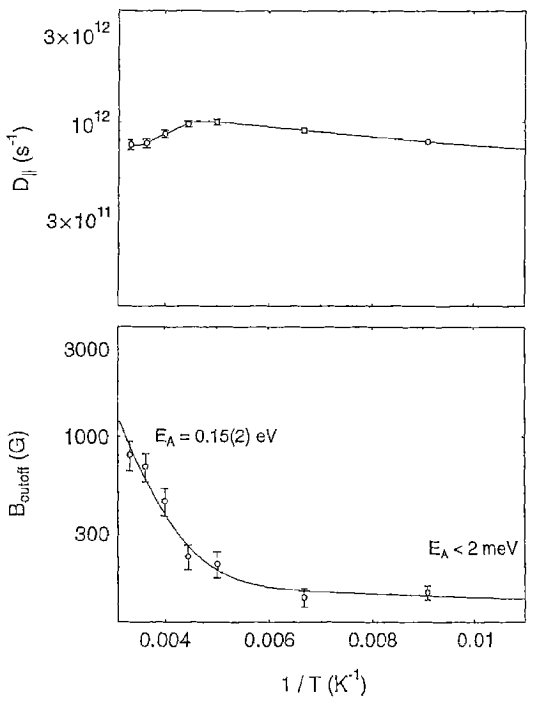

Fig. 3. The upper plot shows the temperature dependence of the inter-site diffusion rate of electron(s) in cytochrome c derived from the B^{-1} dependent part of the relaxation parameter. The lower plot shows the cutoff field determined in Fig. 2 against inverse temperature, from which dominant activation-type energies can be determined in diffrent temperature regions.

Microscopic aspects of electron transfer in cytochrome c revealed here

All of these results lead to the following microscopic picture of the electron transfer processes for the muon-injected electron in cytochrome c.

(1) An electron brought in by the μ^+ and liberated at a bonding site initially makes a topologically linear motion along the chain of the cytochrome c.

(2) The inter-site diffusion rate along the 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,[7,9] becomes a value of the order of 10^{12} rad/s and almost temperature-independent (Fig. 3). This value is encouragingly consistent with those obtained using optical methods in Ruthenated proteins for short electron transfer distances in the region of 5 Å.[15]

(3) The cutoff process represents departure on a longer timescale from the topologically one-dimensional diffusion which occurs on the shorter timescale. Depending upon the temperature region, the cutoff field is dominated by two different processes as seen in the temperature dependence (Fig. 3); 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 the cutoff field seems to be related to the well-known structural change of some proteins which has been suggested as glass-like transition.[16] A naive picture based upon previous μ^+SR studies on high molecular weight conducting polymers[5-7] would suggest an increase in the effective dimensionality of the diffusion at temperatures higher than 200 K due to an increased inter-chain diffusion rate. In the context of a protein such as cytochrome c with coils and folds in its structure, the "inter-chain" diffusion might perhaps be interpreted as "inner-loop" jumps, which could well be strongly activated by the increased thermal displacement of the polymer occuring above the glass transition.

The most important unknown factors in the present μ^+SR studies are the distribution of locations of the μ^+ bonding-sites with corresponding uncertainty in the electronic structure of the μ^+ and the site from where the electron starts its linear motion. At this stage of our μSR studies, there is little information on these questions. In this respect, the lower field relaxation part seen in Γ for B_{ext} below the crossover might provide some key informations; if it is sensitive to the magnetism of the Fe ions situated at the center of cytrochrome c then, by measuring a precise value of the hyperfine interaction parameter between the stationary μ^+ and the Fe ion (and its temperature as well as angular dependence), information about the μ^+ location may be revealed. For this purpose, muon spin RF resonance as well as level crossing resonance will be most helpful. These experiments are now in progress.

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 cythochrome c was directly detected in the present experiment. The present μSR method should be contrasted with the experiments using photo-excited electrons, where electron transfer was measured through a path connecting between heme-Fe and Ru-susbstituted portion (bis (2,2'-bipyridine) imidazole).[1] It should also be contrasted with the electron thansfer measurements within a zinc-substituted protein initiated by flash photo-production,[17] where electron transfer through a path

connecting heme-centers is defected.

The μ^+SR measurement, where high efficiency of the measurements should be emphasized (typically 5 min for a μSR spectrum shown in Fig. 1), is easily able to be extended to cytochrome c in various chemical and biological environments; e.g. in solution with different values of pH, etc. It would be interesting to conduct similar experiments on other related proteins, such as 1) as suggested by N. Go, Lysozyme without Fe ions where no electron transfer is expected and 2) as suggested by M. Ataka and T. Kubota, cytochrome c with Fe(2+) ions where electron transfer is somewhat suppressed. Preliminary measurements on these systems have given some supporting results, details of which will be reported elesewhere.

The highly sensitive μSR method, which was applied for the first time to the microscopic nature of the electron transfer process in a protein, may have potential application in a wide-variety of fields. Most importantly, because of original high energy nature of the probe, this method can be applied to a protein *in vivo*. As one example for the future, it might be possible to use the technique to obtain new information on the basic functions of brain activity.

We acknowledge helpful discussions with Professor N. Go and Drs. M. Ataka, T. Kubota, N. Igarashi, T. Iizuka and A. Kira. The authors would like to thank Professors A. Arima, M. Oda and other related persons at RIKEN and Drs. P. Williams, A. D. Taylor and W. G. Williams and other related persons at RAL for their kind support and encouragement in the developments of the RIKEN-RAL Muon Facility. Helpful discussions with Professors K. Kehr and T. P. Das on condensed-matter related problems are also acknowledged.

References

1) T. B. Karpishin, M. W. Grinstaff, S. Komer-Panicucci, G. Mclendon and H. B. Gray: Structure **2**, 415 (1994).
2) G. M. Pettigrew and G. R. Moor: *Cytochrome c -Biological Aspects* (Springer-Verlag, Berlin-Heidelberg-New York-London-Paris-Tokyo, 1987).
3) R. A. Scott and A. G. Mauk: *Cytochrome c -A multidisciplinary approach* (University Science Books, Sausalito, California, 1995).
4) R. E. Dickerson, M. L. Kopka, C. L. Borders, Jr., J. Varnum, and J. E. Weinzierl: J. Mol. Biol. **29**, 77 (1967).
5) K. Nagamine, K. Ishida, T. Matsuzaki, K. Nishiyama, Y. Kuno, T. Yamazaki, and H. Shirakawa: Phys. Rev. Lett. **53**, 1763 (1984).
6) K. Ishida, K. Nagamine, T. Matsuzaki, Y. Kuno, T. Yamazaki, E. Torikai, H. Shirakawa, and J. H. Brewer: Phys. Rev. Lett. **55**, 2209 (1985).
7) F. L. Pratt, S. J. Blundell, W. Hayes, K. Nagamine, K. Ishida, and A. P. Monkman: Phys. Rev. Lett. **79**, 2855 (1997).
8) M. A. Butler, L. R. Walker, and Z. G. Soos: J. Chem. Phys. **64**, 3592 (1976).
9) R. Risch and K. W. Kehr: Phys. Rev. B **46**, 5246 (1992).
10) K. Nagamine: Invited talk at Gordon Conference on Rapid Electron Transfer in Macro-Molecule (Los Angels, March 1987).
11) C. Bucci, P. R. Crippa, G. M. de'Munari, G. Manfredi, M. Tedeschi, and A. Vecli: Hyperfine Interact. **6**, 425 (1979).
12) K. Nagamine, T. Matsuzaki, K. Ishida, I. Watanabe, S. N. Nakamura, R. Kadono, N. Kawamura, S. Sakamoto, M. Iwasaki, M. Tanase, M. Kato, K. Kurosawa, G. H. Eaton, H. J. Jones, G. Thomas, and W. G. Williams: Hyperfine Interact. **102**, 521 (1996).
13) K. Nagamine: Hyperfine Interact. **8**, 787 (1980).
14) K. Nagamine: Z. Phys. C **56**, S215 (1992).
15) C. C. Moser, J. M. Keske, K. Warncke, R. S. Farid, and P. L. Dutton: Nature **355**, 796 (1992).
16) For the typical earlier reference, F. Parak, E. N. Frolov, R. L. Mossbauer, and V. I. Goldanskii: J. Mol. Biol. **145**, 825 (1981).
17) S. E. Peterson-Kennedy, J. L. McGourty, J. A. Kalweit, and B. M. Hoffman: J. Am. Chem. Soc. **108**, 1739 (1986).