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2004 J. Phys.: Condens. Matter 16 S4797

(http://iopscience.iop.org/0953-8984/16/40/020)

INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **16** (2004) S4797–S4806 PII: S0953-8984(04)84850-1

Electron transfer in proteins and DNA probed by muon spin relaxation

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Received 6 April 2004
Published 24 September 2004
Online at stacks.iop.org/JPhysCM/16/S4797
doi:10.1088/0953-8984/16/40/020

Abstract

Electron-transfer phenomena in biological macromolecules are among the most important processes of life science. So far, a limited number of microscopic studies exist, and most knowledge is based on macroscopic studies. In order to overcome this situation, a labelled electron method with positive muons was recently developed and successfully applied to directly explore microscopic electron-transfer phenomena in representative proteins, such as cytochrome c, myoglobin and cytochrome c oxidase, and DNA. The principle, some details of each experiment and future perspectives are described.

1. Introduction: labelled electron method for muon life science

By using muons, various types of scientific research, both fundamental and applied, are now extensively progressing (Nagamine 2003a). At the same time, intense hadron accelerators at the level of MW or stronger are either under construction or under planning around the world, not only for spallation neutron sources, but also for neutrino factories. There, intense muon beams become available, providing opportunities for the further development of muon science. Among various topics in scientific research with muons, life science has been left almost untouched until recently. Some breakthrough experiments have been undertaken to explore electron transfer in biological macromolecules.

The electron-transfer process in macromolecules, such as proteins, is an important part of many biological phenomena, such as the storage and consummation of energy and photosynthesis. A number of experimental investigations have been carried out to explore 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 transfer, it is thus very important to use methods that provide information at a more microscopic level.

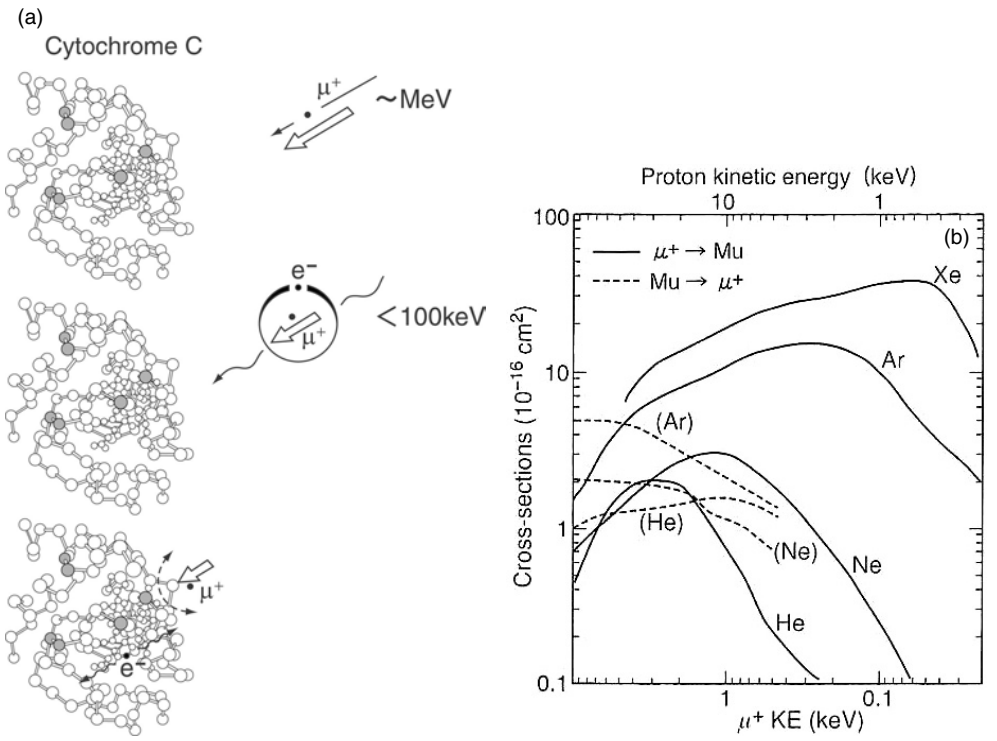

Figure 1. (a) Schematic picture of the role of positive muons for studies of electron transfer in macromolecules. Energetic μ^+ introduction (top), a process of electron pick-up to form Mu during μ^+ slowing-down (middle) and thermalization and physical/chemical bonding of the μ^+ and/or Mu and a release of the brought-in electron (bottom). (b) Charge-exchange total cross section for muons at various kinetic energies (KE), as calculated for He, Ne, Ar and Xe (Brewer and Crowe 1978, Walker 1983). The solid curves show electron capture and the dashed ones electron loss.

Recently, by extending muon spin-relaxation spectroscopy, a method to directly observe microscopic aspects of electron transfer has been proposed and successfully established (Nagamine *et al* 2000). As for electron transfer in a biological macromolecule, the muon spin-relaxation method offers great potential (see figure 1(a)). During the slowing-down process in soft materials, such as biological macromolecules, the injected μ^+ has preferentially a chance to pick up one electron to form a neutral atomic state of a muonium, as indicated in the charge-exchange total cross section for muons in an isolated inert gas molecule (see figure 1(b)). This muonium is then thermalized, followed by chemical bonding to a reactive site on the molecule. Then, depending upon the nature of the molecule and the molecular binding, 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 the electron movement, can be sensitively detected by measuring the spin-relaxation process of the μ^+ using the μSR method, which occurs through a magnetic interaction between the μ^+ spin and the spin of a moving electron produced by the μ^+ itself. In other words, by introducing an 'electron' and an 'electron observer' at the same time, a tracer of the 'electron' can be made: the labelled electron method.

We must note that the introduction of an electron in the form of an energetic Mu is, at this stage of understanding, an assumption. Now, the so-called radiolysis effect, where a muonium

is formed between a thermalized μ^+ and an electron produced in the radiation track, is known to be the other major mechanism of muonium formation. Therefore, one should experimentally check the validity of the assumption by e.g. applying an electric field etc. The other possibility of the role of μ^+ introduction into a biological macromolecule is participation of the μ^+ in a biological process, like the H^+ pumping mechanism. These other possible mechanisms, once known to be effective, may open other possible life-science studies with muons.

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 spin of the moving electron and that of the stationary muon, the characteristic dimensionality of the electron motion can be studied by observing the field dependence of the muon spin-relaxation rate (λ_μ), with reference to the strength of the applied magnetic field ($B_{\rm ext}$) (Butler *et al* 1976): 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 usually have a significant $B_{\rm ext}$ dependence. Progress has been made in a theoretical understanding of this paramagnetic relaxation process by Risch and Kehr, who considered a direct stochastic treatment of the random-walk process of a spin that is rapidly diffusing along a topologically one-dimensional chain (Risch and Kehr 1992). An error-function type longitudinal relaxation function (hereafter called the RK function), $G(t) = \exp(\Gamma t)\mathrm{erfc}(\Gamma t)^{1/2}$, was proposed for $\nu t_{\rm max} \gg 1$, where ν is the electron spin-flip rate, $t_{\rm max}$ the experimental timescale and Γ a relaxation parameter. In this theoretical treatment, in the case of topologically one-dimensional electron motion, Γ is proportional to $1/B_{\rm ext}$. Recently, the RK theoretical model was analysed and shown to be helpful for the purpose of experimenters (Pratt 2004). It was confirmed that $\Gamma \propto 1/B_{\rm ext}$ holds for $\gamma > \omega_0 > \nu$, where γ is the on-chain diffusion rate ($D_\parallel$) of an electron and ω_0 is the hyperfine coupling constant between a μ^+ and an electron at the closest distance. There, Γ becomes $\omega_0^4/2\omega_{\rm e}\gamma^2$, where $\omega_{\rm e} = \gamma_\mu B_{\rm ext}$.

This idea of the sensitive detection of the electron behaviour in macromolecules using muons has been successfully applied in studies of electron transport in conducting polymers. A soliton-like motion of the μ^+-produced electron in *trans*-polyacetylene has been observed, which is in contrast with localization of that electron in *cis*-polyacetylene such as the formation of a radical state (Nagamine *et al* 1984, Ishida *et al* 1985). In a succeeding experiment on polyaniline, the usefulness of the RK function was confirmed experimentally for the polaronic motion of conduction electrons in polyaniline, where a way to obtain 1D- and 3D-diffusion rates from the fitted RK functions was pointed out (Pratt *et al* 1997). More recent achievements have been reported (Pratt 2004).

2. Probing electron transfer in cytochrome c and other proteins

In order to obtain microscopic information on electron transfer in biological macromolecules, like proteins or DNA, the labelled electron method offers great potential. Depending upon the nature of the molecule, the electron accompanying the μ^+ into the molecule can have a variety of characteristic behaviours, such as a linear motion along the molecular chain, localization to form a radical state etc. These behaviours, as mentioned above, can be studied with high sensitivity by measuring the spin-relaxation process of the μ^+ using the μSR method.

Among a large number of proteins in existence, cytochrome c is one which has attracted much attention, since it plays an essential role in the respiratory electron-transport chain in mitochondria; it holds a position next to the site of the final process of the cycle and transfers electrons to the surrounding oxidase complex. The crystal of cytochrome c is commercially available and its crystal structure has been well studied by x-ray measurements.

The first series of experiments on μ^+ relaxation in cytochrome c were conducted using an intense pulsed beam of 4 MeV μ^+ at port II of the RIKEN-RAL muon facility (Nagamine *et al* 2000, Nagamine 2002, 2003b). The cytochrome c used here was the Fe^{3+} type in a polycrystalline powder form, extracted from horse heart (Wako-Chemical product). As references, cytochrome c with Fe^{2+}, prepared by Drs Ataka and Kubota, and lysozyme extracted from chicken egg (Sigma) were used, both of which are known not to have any electron-transfer behaviour.

The μ^+SR measurements were carried out for a range of temperatures between 5 and 300 K and for a number of longitudinal magnetic fields in the range of 0–0.4 T. All measurements were conducted on an as-received powder sample. At each of the measurement temperatures, the μ^+ relaxation function was found to have an external field dependence (figure 2). The observed relaxation functions ($G(t)$) were fitted with the RK function, whereupon the longitudinal relaxation parameter (Γ) obtained at various temperatures was found to decrease monotonically with increasing $B_{\rm ext}$. Upon a closer inspection of the $B_{\rm ext}$ dependence of Γ, two separate field regions seemed to be found, exhibiting different behaviour: (1) a region of weak field dependence (lower field) and (2) a $(B_{\rm ext})^{-1}$-dependence region (higher field) (figure 2). The latter region exhibited the characteristic μ^+ spin relaxation behaviour due to the linear motion of a paramagnetic electron. Similar measurements made for both cytochrome c with Fe^{2+} and lysozyme did not show any region where Γ follows a $(B_{\rm ext})^{-1}$ dependence. The critical cut-off field where the second type of behaviour supersedes the first one has significant temperature dependence: it grows smaller with decreasing temperature. It can further be seen that the temperature dependence of the cut-off field can be represented by the sum of two activated components of the form $\exp(-E_{\rm a}/kT)$, where $E_{\rm a}$ is the activation energy; of these, one has an activation energy of 150 meV (dominant above 200 K), while the other has an activation energy of less than 2 meV (dominant below 200 K). 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 'inter-loop' jumps, which could well be strongly activated by the increased thermal displacement of the polymer occurring above the glass transition temperature of 200 K.

The most important unknown factors in the present μ^+SR studies are the distribution of the locations of the μ^+ bonding sites, together with the corresponding uncertainty in the electronic structure of the μ^+ and the site from which the electron commences its linear motion. For the purpose of elucidating these matters, the muon spin RF resonance and the level crossing resonance will be the most helpful techniques. Preliminary results gave about a 10 ppm paramagnetic shift with a characteristic temperature dependence, which will provide information on the distance of the μ^+ from the haem Fe. Theoretical studies on the possible μ^+ and Mu sites in cytochrome c have been carried out, suggesting that they exist at the nitrogen of the pyrrole ring and/or negatively charged parts of amino acids (Cammarere *et al* 2000).

Experiments have been extended to other crystallized proteins such as (1) myoglobin, which is known to be important in oxygen transport with a molecular structure similar to that of an electron-transfer protein, like cytochrome c, and (2) cytochrome c oxydase, which is known to stay at the terminal position of the electron transfer in mitocondria aspiration cycle.

As for the myoglobin experiment, we used a crystal extracted from horse muscle (Sigma). The characteristic difference in the temperature dependence in the inter-chain diffusion rate was seen between cytochrome c and myoglobin, suggesting a difference between 'natural' and 'artificial' electron transfer in the protein as seen in figure 3 (Nagamine *et al* 2000).

The experiment on cytochrome c oxidase was conducted in collaboration with Drs Yoshikawa and Shinzawa-Ito of Himeji Institute of Technology and Tsukihara of the Institute for Protein Research, Osaka University. Structure studies have been carried out using high-resolution x-rays (Tsukihara *et al* 1995). Due to a difficulty in sample preparation, the

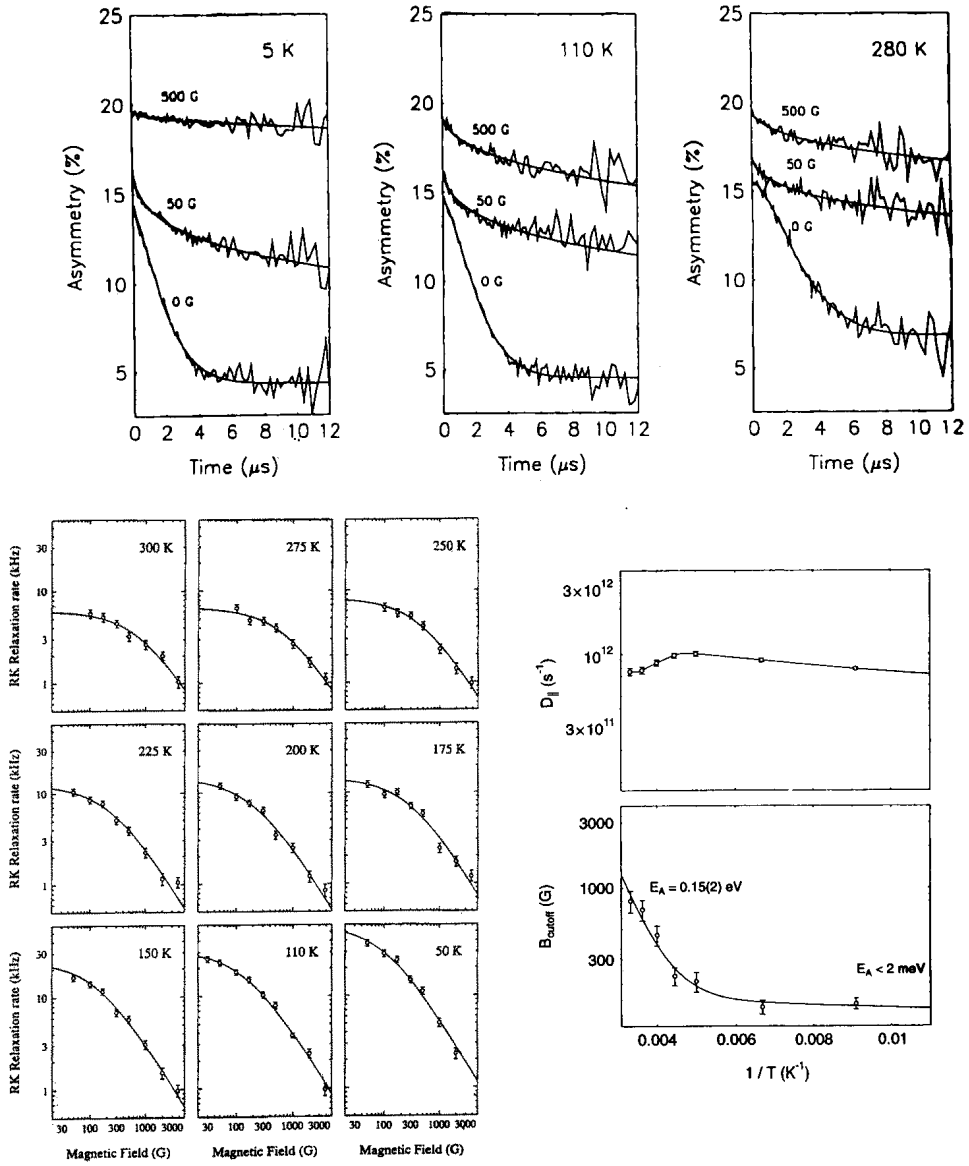

Figure 2. Typical μ^+ spin-relaxation time spectra in cytochrome c at 5, 110 and 280 K under external longitudinal fields of 0, 50 and 500 G (above). For a finite field the curves show best fits using the RK function. The RK relaxation parameter (Γ) is given versus the external longitudinal magnetic field for the μ^+ in cytochrome c at various temperatures (below). The $(B_{\rm ext})^{-1}$ dependence part can be seen to become significant in the higher field region, and the critical field (cut-off field) for the onset of the $(B_{\rm ext})^{-1}$ dependence can be seen to have a clear temperature dependence. The data are taken from the work of Nagamine *et al* (2000).

actual sample was composed of bovine heart cytochrome c oxidase (50%) and the surfactant material Deyl-β-D-maltoside and buffer chemicals of NaH_2PO_4 (altogether 50%).

(1) At room temperature, electron transfer along the chain in cytochrome c oxidase is very much suppressed compared to that in cytochrome c and myoglobin.

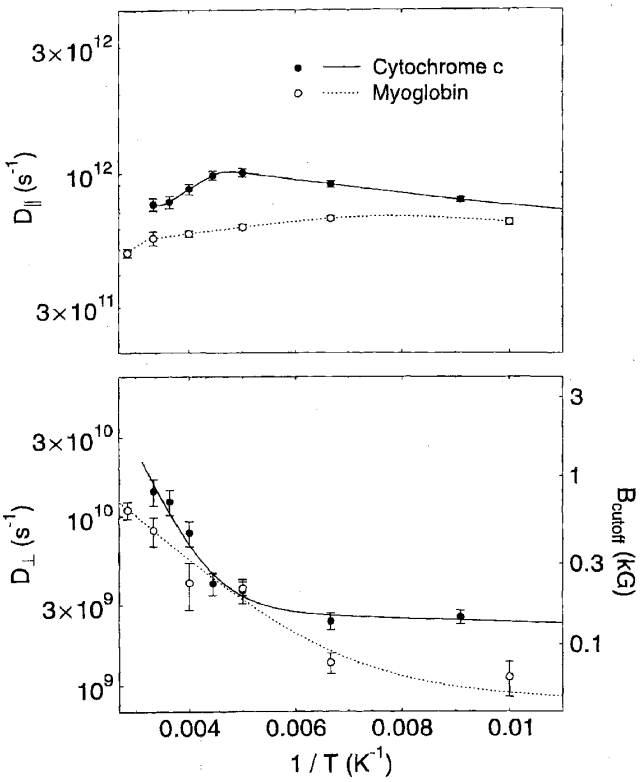

Figure 3. Upper plot showing the temperature dependence of the parallel diffusion rate of an electron in cytochrome c and myoglobin derived from the B^{-1}-dependent part of the relaxation curve. The lower plot shows the perpendicular diffusion rate, derived from the cut-off field determined in the Γ versus B data, plotted against the inverse temperature. The data are taken from the work of Nagamine *et al* (2000).

(2) By reducing the temperature, the electron transfer along the chain becomes evident, particularly below 150 K; Γ takes an inverse-field dependence.

The results may contain the contribution of signals from either the surfactant or the buffer. Since there exist several haem centres in cytochrome c oxidase, there might be several corresponding signal components.

Recently, the labelled electron method was applied to cytochrome c in a pH-controlled water solution (Ikedo and Nagamine 2004). The measurement was intended to be extended to the dependence of the intra- and inter-electron transfer rate on the pH of the solution. As the very beginning stage, it was discovered that the μ^+ relaxation under zero external field evidently seen in crystalline cytochrome c at room temperature becomes suppressed for that dissolved in a water of pH $= 6$ in the concentration range at around 50%. Systematic studies are now in progress.

3. Probing electron transfer in DNA

The labelled electron method was also applied to DNA. Electron-transfer phenomena in DNA are known to be important for not only understanding the damage and repair mechanisms,

but also for possible applications to new bio-devices. The experimental finding of a possible electron transfer between G (guanine) bases by biochemical approaches using metal-modified DNA (Meggers *et al* 1998) has accelerated both experimental and theoretical studies. The U. Yamanashi–RIKEN–KEK–Oxford–Tokyo Kasei Gakuen U.–Juelich collaboration has conducted a μSR experiment at RIKEN-RAL on crystalline DNA in both A and B conformations of the DNA extracted from calf thymus (Torikai *et al* 2001). Both fibrous Na-DNA samples in A and B forms and oriented Li-DNA films were used. The fibrous DNA specimens were extracted from calf thymus with sodium salt and purchased from Calbiochem under a standard biological sample preparation. The DNA fibres were hydrated to 75% and 95% relative humidity to form the crystalline A and B conformation, respectively (see figure 4). The conformation of each sample has been determined by the low-frequency Raman scattering method. To maintain hydration during the experiment, hence the crystalline conformation of each sample, they were then contained in sample cases made of Teflon with mica windows of 25×25 mm^2. An oriented Li-DNA film of 50×50 mm^2 in area was prepared by the wet spinning method from calf thymus DNA fibres, and was hydrated to 75% to form the crystalline B form (Grimm and Rupprecht 1989).

As shown in figure 4, after analysing μSR data using the Risch–Kehr function, the relaxation parameters (Γ) were found to take an inverse-field dependence in both the A and B forms above 80 G, suggesting the existence of a quasi-1D rapid diffusion of electrons in DNA strands. However, there is a clear difference in the field dependence below 80 G: a continuation of the inverse-field dependence in the A form, while a clear peak and dip in the B form. The result demonstrates that some aspects of the quasi-1D diffusion seen in the labelled electron method do correlate with the geometrical arrangements of the base pairs. The result suggests the importance of a picture of electron transfer through the base pairs. In the B form, both fibrous Na-DNA and oriented Li-DNA film showed essentially similar field dependence.

By using the high-field data above 80 G, exhibiting $\Gamma \propto 1/B$, one can obtain the diffusion rate of the electron labelled by the μ^+ through DNA. Admitting the Risch–Kehr formula, the electron diffusion rate in topological one-dimensional motion ($D_\parallel$) can be written as $D_\parallel \cong [(\omega_0^4/2\omega_e)(1/\Gamma)]^{1/2}$ (for $D_\parallel \gg \omega_0^2/\sqrt{(2\omega_e\nu)}$, ν: electron spin flip rate), where ω_0 is the muon–electron hf coupling at the closest approach, ω_e is the electronic Larmor frequency and Γ is the RK relaxation parameter. There, each value can be estimated as follows: ω_0 can be tentatively obtained by the recovery of the initial asymmetry against the field (decoupling), like $2\pi \times 350$ MHz. Thus, by taking a Γ of 1.0×10^4 Hz at $B_{ext} = 1000$ G, and an ω_e of 1.76×10^{10} Hz at $B_{ext} = 1000$ G, one can obtain $D_\parallel$(DNA) $\cong 2.5 \times 10^{11}$ rad s^{-1}, which should be compared to the $D_\parallel$(protein) $\cong 1 \times 10^{12}$ rad s^{-1}, as shown in figure 3.

The low-field data that demonstrate contrasting field dependence between the A and B forms can be interpreted according to the following pictures. (i) Following the arguments made for a protein, as enhanced 3D electron diffusion in the B form, while enhanced 1D electron diffusion in the A form. (ii) The low-field data can be seen in the field dependence with a peak ($\sim$80 G) and a dip (10 G) in the B form, while none of the structure in the A form, suggesting a level-crossing resonance-type effect, namely (ω_μ) $\cong$ (ω_{hfs} in the surrounding system) at $\sim$70 G.

The field-dependence measurements on both A- and B-form DNA were extended at typical temperatures between 160 and 353 K. Typical examples of the field dependence at various temperatures above 260 K observed for the B form are shown in figure 5. Around room temperature, as T becomes higher, Γ (of the $1/B$ dependence) becomes higher (e.g. Γ (100 G) at 260 K is 0.12×10^6, while Γ (100 G) at 280 K is 0.42×10^6), namely $D_\parallel$ becomes slower. The apparent decrease of $D_\parallel$ against temperatures above 260 K is in contradiction to the results obtained in λ-phage DNA measured by contactless conductivity measurements of

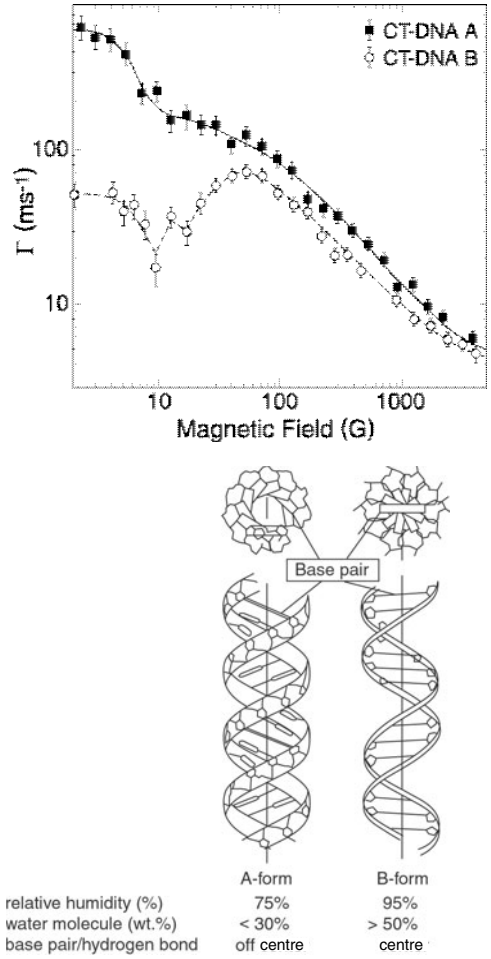

Figure 4. Field dependence of the observed relaxation parameters in both A form and B form of the DNA (upper) and crystalline structure of DNA in A form (lower left) and B form (lower right) with a difference in relative humidity, water molecule concentration (wt%) and base pair/hydrogen bond.

microwave reflection and absorption, where λ-phage DNA, both dry and in buffer, was found to be insulating below 200 K; the conductivity increased exponentially with temperature increase above 250 K (Tran *et al* 2000). Activation-type models, such as the thermal hopping of charges between G–C base pairs, do not explain the present μSR results. The influence of molecular dynamics, such as breathing of base pairs and conformational fluctuation, should also be considered.

Below 260 K down to 200 K, on the other hand, a drastic change of the μ^+ spin relaxation was observed for both A- and B-form DNA in low magnetic field, suggesting the critical slowing-down corresponding to the glassy dynamics (Sakolov *et al* 1999). At 160 K, the μ^+ spin relaxation is well described by the dynamical Kubo–Toyabe function up to around 30 G; the Risch–Kehr function, well explaining the observed μ^+ spin relaxation above 260 K, does not reproduce the data at low temperatures below 260 K. In high magnetic field above 1 kG, the μ^+ spin relaxation rate is very small and field independent in the observed time range. These aspects suggest the significant suppression of electron mobility in the glass phase of DNA.

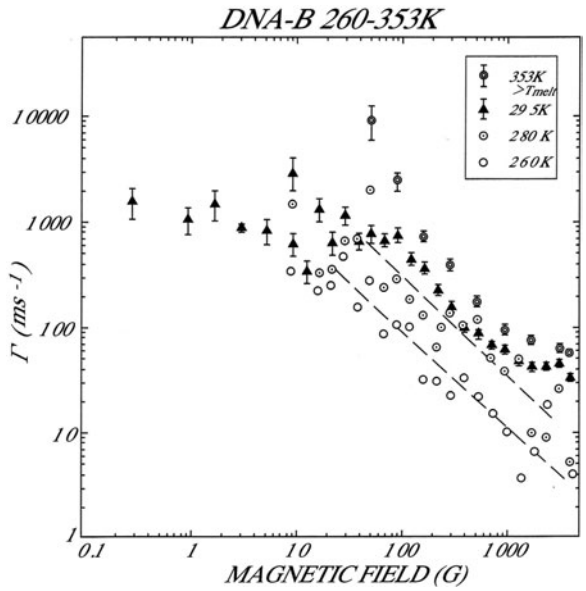

Figure 5. Relaxation parameters versus the magnetic field at various temperatures around room temperature for B-form DNA with an emphasis on the $1/B$ dependence by a straight line.

Below 200 K, the formation of a muonium-like state was observed in both the A and B forms. The spectroscopic data for the muonium-like state, namely, the hyperfine coupling constant, was investigated by the muon spin rf resonance method: 46 MHz at RIKEN-RAL and 473.5 MHz at KEK-MSL. The amplitude of the observed muonium signals corresponded to 20% of the diamagnetic signal at room temperature. So far, close to the vacuum value has been obtained for the hyperfine coupling constant. Precise measurement to find difference in the muonium hfs constant between the A form and the B form is now in progress. Theoretical prediction of the difference was carried out for the muonium at the base plate of the adenine (Scheicher and Das 2004).

For further discussions, we need knowledge about the stopping site of muons that emit and probe the labelled electrons. As reported separately, first-principles electronic-structure investigations were made for both the binding energies and the hyperfine interactions of the trapped Mu in the six-member ring in adenine, regarding the dependence on the A and B conformations in terms of the packing densities (Scheicher and Das 2004). The result suggests a strong influence on the A and B conformations. This effect might be related to what we observed in the low-field region at around room temperature as seen in figure 4.

4. Future perspectives

The μ^+SR method as described above, where the high efficiency of the technique should be emphasized, can be easily extended to the study of proteins or DNA in various chemical and biological environments. Most importantly, because of the initial high-energy nature of the probe, this method can be applied to proteins or DNA *in vivo*. As one of the future dreams, it might be possible to use the present technique to obtain some aspects of electron transfer in a part of active brain of the human body which might be related to new information on the basic functions of the brain activity.

At the same time, because of an inherent difficulty in the crystallization of proteins or DNA, the size of a standard new crystal of biological macromolecule is very small, 100 μm or less. In order to apply the labelled electron method for these small-size crystals, the development of a μ^+ micro-beam is indispensable. The idea of a muon micro-beam by accelerating an ultra-slow μ^+ beam is presented in a recent publication (Nagamine 2003c).

Acknowledgments

The authors acknowledge contributions by the following persons to the pioneering μSR experimental research on life-science subjects: Drs F L Pratt, I Watanabe, S Ohira, Y Ikedo and K Nishiyama. Acknowledgements should be given concerning the help of sample preparation and analysis done by Drs M Ataka, T Kubota and N Igarashi for cytochrome c, Professors T Tsukihara and S Yoshikawa for cytochrome c oxidase and H Urabe and H Grimm for DNA. Helpful discussions on biological electron transfer with Professors N Go, the late K Kehr, T Iizuka and A Kira are also acknowledged. We also acknowledge theoretical work on μSR location in proteins and DNA by Professor T P Das, Mr R Scheicher and other collaborators at the State University of New York at Albany. The present experimental work was conducted at mostly RIKEN-RAL and KEK-IMSS-MSL. We acknowledge Professors M Oda, A Arima, S Kobayashi, M Iwasaki and other related persons at RIKEN, Drs P Williams, A D Taylor, W G Williams and other related persons at RAL and Professors H Sugawara, Y Kimura, A Koma, Y Totsuka and other related persons at KEK. The work was partly supported by a Grant-in-Aid for Fundamental Research by JSPS.

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