Use of Slow Muons in the Life Sciences; Present and Future

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

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1. Introduction; Muon Life Science by the Labelled Electron Method

By using muons, various types of scientific researches, both fundamental and applied, are now extensively in progress (Nagamine 2002c). At the same time, intense hadron accelerators at the level of MW or stronger are either under construction or under planning in the world, not only for neutrino factory or spallation neutron source. There, the intense muon beam becomes available, providing opportunities of the further development of the muon science. Among various topics in scientific researches with muon, the life science has been left untouched until recently. Some break-through experiments have been undertaken to explore electron transfer in biological macro-molecules.

The electron-transfer process in macro-molecules, such as proteins, is an important part of many biological phenomena, such as the storage and consummation of energy and photo-synthesis. 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 the 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 established a method to directly observe microscopic aspects of electron transfer. The principle of μSR is based upon polarization phenomena in both muon production and muon decay. The spin of the μ^+, when it is born by the decay of a π^+, 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 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 and the e^+ takes a spacial 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. 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 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^+.

In order to obtain microscopic information about electron transfer in a biological macro-molecule, the muon spin-relaxation (μSR) method offers great potential (see, Fig. 1). During the slowing-down process, the injected μ^+ picks up one electron to form a neutral atomic state of 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, by introducing "electron" and an "electron observer" at the same time, the tracer of the "electron" can be made: the labelled-electron method.

We must note that the introduction of an electron in the form of energetic Mu is, at this moment, an assumption. Since the other mechanism of radiolysis effect is known to be the other major mechanism, one should check experimentally the validity of the assumption by e.g. applying an electric field. The other possibility of the role of the μ^+ introduction into biological macro-molecule is a participation of the μ^+ in biological process like H^+ pumping mechanism. These mechanisms other than the Mu introduction, once known to be effective, may open the 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 moving electrons and the stationary muons, the characteristic dimensionality of the electron motion can be studied by varying the externally applied magnetic field (B_{ext}) and observing the dependence of the muon spin relaxation rate (λ_μ); for one-dimensional electron motion $\lambda_\mu \propto (B_{ext})^{-1/2}$, for two-dimensional electron motion $\lambda_\mu \propto (\alpha - log\beta B_{ext})$, where α and β are constants, and for three-dimensional electron motion λ_μ does not usually have significant B_{ext} dependence (Butler 1976). 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 (Risch and Kehr 1992). 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 $\lambda t_{max} \gg 1$, where λ is the electron spin flip rate, t_{max} the experimental time scale and Γ a relaxation parameter. In this theoretical treatment, in the case of topologically one-dimensional electron motion, Γ is proportional to $1/B_{ext}$. This idea of the sensitive detection of the electron behaviour in macromolecules using muons has been applied successfully in studies of the electron transport in conducting polymers. A soliton-like motion of the μ^+-produced electron in trans-polyacetylene has been observed, which contrasts with the localization seen in cis-polyacetylene following the formation of a radical state (Nagamine 1984, Ishida 1985).

In the succeeding experiment on polyaniline, the usefulness of the R-K function was confirmed experimentally for the polaronic motion of conduction electrons in polyaniline, where the way how to obtain 1D- and 3D-diffusion rates from the fitted R-K functions was pointed out (Pratt 1997).

2. Probing Electron Transfer in Proteins

In order to obtain microscopic information on electron transfer in biological macromolecules like proteins or DNA, the labelled electron method offers a great potential. Depending upon the nature of the molecule, the electron accompanying the μ^+ into the molecule can have a variety of characteristic behaviours including localization to form a radical state, a linear motion along the molecular chain 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.

Of the very large number of proteins in existence, cytochrome c is the 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. Experiments on the μ^+ relaxation in cytochrome c were conducted using an intense pulsed beam of 4 MeV μ^+ at RIKEN-RAL (Nagamine 2000, 2002a).

At each of the measurement temperatures, the μ^+ relaxation function was found to have an external field dependence (Fig. 2). The observed relaxation functions G(t) were fitted with the R-K function, whereupon the longitudinal relaxation parameter Γ obtained at various temperatures was found to decrease monotonically with increasing B_{ext}. On closer inspection of the B_{ext} dependence of Γ, there seem to be two separate field regions exhibiting different behaviour, (1) a region of weak field-dependence (lower field) and (2) a $(B_{ext})^{-1}$ dependence region (higher field) (Fig. 2). The latter region exhibits the characteristic μ^+ spin relaxation behaviour due to the linear motion of a paramagnetic electron. The critical field of the cutoff field where the second type of behaviour supersedes the first one has a significant temperature dependence: it grows smaller with decreasing temperature. It can further be seen that the temperature dependence of the cutoff field can be represented by the sum of two activated components of the form $\exp(-E_a/kT)$, where E_a is an 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 locations of the μ^+ bonding-sites, together with the corresponding uncertainty in the electronic structure of the μ^+ and the site from where the electron commences its linear motion. For the purpose of elucidating these matters, muon spin RF resonance and level crossing resonance will be the most helpful techniques. Theoretical studies on the possible μ^+ and Mu sites in cytochrome c have been carried out, suggesting at the nitrogen of the pyrrole ring and/or negatively charged parts of amino acids (Cammarere 2000).

Experiments have been extended to other proteins such as 1) myoglobin which is known to be important in oxygen transport with a molecular structure similar to that of the electron-transfer protein and 2) cytochrome c oxydase which is known to stay at the terminal position of electron transfer in mitocondria aspiration cycle. Characteristic difference in temperature dependence in inter-chain diffusion rate was seen between cytochrome c and myoglobin, suggesting a difference between "natural" and "artificial" electron transfer in protein (Fig. 3). Also, a limited electron transfer at room temperature, while an enhanced one at low temperature was seen in cytochrome c oxydase suggesting a possible contribution to explore electron transfer path.

3. Probing Electron Transfer in DNA

The labelled electron method was also applied to DNA. Electron-transfer phenomena in DNA is known to be important because of not only for understanding damage and repair mechanisms, but also for possible applications to new bio-devices. The experimental finding of a possible electron transfer between G (Guanin) bases has accelerated both experimental and theoretical studies. The Yamanashi U-RIKEN-KEK-Oxford-Tokyo Kasei Gakuen U.-Juelich collaboration has successfully conducted a μSR experiment at RIKEN-RAL on an oriented DNA in both A and B conformations of the DNA extracted from calf thymus (Fig. 4), and observed electron transfer in DNA at room temperature (Torikai 2002). There, after analyzing μSR, data along the same procedure mentioned above, the relaxation parameters Γ were found to take an inverse-field dependence in both A and B cases above 80 G (Fig. 4), suggesting an existence of quasi-1D rapid diffusion of electrons in DNA. However, there is a clear difference in the field dependence below 80 G; a continuation of the inverse-field dependence in the A case, while no field dependence in the B case. The result demonstrates that some aspects of the quasi-1D diffusion seen in the labelled electron method does correlate with the arrangements of base-pairs. The result is somewhat consistent with a picture of electron hopping through base pairs.

4. Future Perspectives

The μ^+SR method as described above, where the high efficiency of the technique should be emphasized, can easily be 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 example for the future, it might be possible to use the technique to obtain new information on the basic functions of brain activity. At the same time, because of an inherent difficulty in crystallization of proteins or DNA, a size of a standard new crystal of biological macromolecule is very small like 100 μm or less. In order to apply the labelled electron method for these small size crystals, the development of μ^+ micro-beam is indispensable. The idea of muon micro-beam is presented in a separate contribution (Nagamine 2002b).

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\begin{figure}
\caption{A schematic picture of the role of positive muons for studies of electron trans
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\begin{figure}
\caption{Typical $\mu^+$ spin relaxation time spectra in cytochrome c at 5 K, 110 K and
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\begin{figure}
\caption{Upper plot showing the temperature dependence of the parallel diffusion rate of
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\caption{Field dependence of the observed relaxation parameters in both A-form and B-for
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