ELSEVIER

Biophysical Chemistry 114 (2005) 149–155

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Improvements in technical assessment and protocol for EPR evaluation of magnetic fields effects on a radical pair reaction

Giancaterino Gualtieri[a,b], Silvia Colacicchi[a,b], Veronica Carnicelli[a], Antonio Di Giulio[a,*]

[a]*Department of Biomedical Sciences and Technologies, University of L'Aquila, L'Aquila, Italy*
[b]*National Institute for Physics of Matter Units of L'Aquila, L'Aquila, Italy*

Received 23 September 2004; received in revised form 8 November 2004; accepted 22 November 2004
Available online 10 December 2004

Abstract

The effects of either static or pulsed magnetic fields on the reaction rate of Fremy's salt–ascorbic acid were studied directly by EPR spectroscopy. Radical pair mechanism (RPM) accounts for the magnetic field effects, but the expected amounts are so small that they need to be observed with particular care with EPR technique. The method is based on the resolution of a pair of EPR signals by the addition of a stationary field gradient, where the signals are coming from the exposed and control capillary sample. To this purpose, a suitable device for the gradient generation was used. Others improvements were the strictly keeping of the same boundary temperature condition in the capillary pairs, obtained by a refrigerating system controlled by a thermocouple, and the use of a pair of Helmholtz coils to generate an external high homogeneous magnetic field. By this experimental set up, we found that the magnetic field induce the decrease of the studied radical reaction rate. This EPR approach is a significant alternative to the spectrophotometric one. Moreover, it offers the advantage to detect both the radicals and/or intermediates involved in the reaction.

Keywords: EPR spectroscopy; RPM (radical pair mechanism); Fremy's salt; Ascorbyl; Ascorbic acid; Gradient coils

1. Introduction

The magnetic field effects represent today a very appealing problem which concerns many people in the world. The main question is if the magnetic field, at low and/or high frequency, is harmful to human health. In spite of 40 years of studies [1] and a very large production of scientific works, it is still very difficult to give a convincing answer to this problem. The reasons are either the conflicting results of scientific research or, particularly, the lack of a reliable theory about a mechanism through which the magnetic field interact with biological systems [2].

Some mechanisms explaining the magnetic field effects were long time ago proposed. Magnetoreception, melatonin depletion, Ca^{2+} binding, ion cyclotron resonance, parametric resonance, other miscellaneous magnetic fields effects and also electric field effects (Ref. [3] and articles therein) are currently accepted theories. Nevertheless, these proposed mechanisms explain only the phenomenological observations. The lack of a fully understood mechanism and of a direct, reproducible and definitive experiment for field-induced effects, resulted in unfocused research, and in inconsistent observations and interpretations [2,4].

Nowadays, it is possible to verify the magnetic field effects on simple chemical systems. In fact, there are no doubts that both static [5] and time-varying magnetic fields [6], can affect the yield and the rate of the free-radicals reactions [7]. For these reactions, magnetic field effects are explained in terms of radical pair mechanism (RPM).

RPM operates when two free radical meet up in presence of a magnetic field to form a pair (RP) [8]. A chemical redox reaction can occur in the pair only if its whole spin function is singlet (S), while if the RP is a triplet (T) the radicals are

* Corresponding author. Dipartimento di Scienze e Tecnologie Biomediche, via Vetoio, I-67010 Coppito-L'Aquila, Italy. Tel.: +39 862 433453; fax: +39 862 433433.
E-mail address: antonio.digiulio@cc.univaq.it (A. Di Giulio).

0301-4622/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2004.11.014

not reactive and they escape in the solution bulk. In some cases, the pair of radicals originated from the breaking of a precursor molecule, and the radicals inherit its spin state, which can be definite. Otherwise, as in our case, RPs are formed by free diffusing radicals in solution and the pairs have a random distribution of their spin functions (25% S and 75% T). These kind of free pairs, or F-pairs, have an overall behaviour as if they were only triplet pairs, because the recombination reaction depletes the solution from the singlet RPs.

S and T energy levels are functions of the magnetic field value, and they are separated by an exchange interaction $2J$, where J depends on the relative distance of the two radicals in the pair. The S state and one of the three triplet components have generally a point of cross over, and during the relative diffusion of the radicals, the energy gap between S and one of the T levels can shrink so that an effective S/T mixing can occur. The mixing have the effect to transfer spin population from one of the non-reactive T energy levels to the chemically reactive state S. The mixing rate moreover depends on the value of the applied magnetic field and on the kind of mechanism typical of the interacting pair (i.e. e. hyperfine interaction or g values) [8]. Consequently, the magnetic field is involved in promoting or preventing the recombination reaction of the radicals.

Only recently, the researchers fairly suggest that RPM might be the lacking mechanism by which an external magnetic field affects biochemical systems. The radicals and radical pairs play very important roles in chemical and biochemical reactions as photosynthesis in plant and in enzymatic reactions in living organisms [8]. This model gives well-defined predictions about the fate of chemical species exposed to magnetic field [4]. "The radical pair mechanism is based upon sound, reproducible and understood science, and it does provide a basic primary way of coupling electromagnetic radiation to the biological system. At such, it is at present unique" [2].

Up to now, many spectrophotometric experiments demonstrated a significant variation in the yield of reaction products under weak static and time-varying magnetic field, using for example the exciplex [9,10]. Nevertheless, in RPM, the radicals involved in the pair are intrinsically paramagnetic and are an expected target for EPR spectroscopy.

The aim of this work was the improvement of the apparatus for direct evaluation, through EPR spectroscopy, of weak static and pulsed magnetic field effects on biological radical reactions, specifically the Fremy's salt–ascorbic acid reaction. This pair of reagents was selected because of the high biological significance of ascorbic acid, one of the more effective blood antioxidants which plays a primary role in biological systems as well as in biochemical processes. In our previous works on this reagent pair, it was observed a long-lasting chemically induced dynamic electron polarization (CIDEP) phenomenon [11]. For this reason, we used the system Fremy's salt–ascorbic acid, which possess the right conditions to verify the radical pair mechanism (RPM), in order to study the phenomenon directly by EPR. Since the expected field effect is very weak, our main improvement was the "parallel detection" of EPR signals from exposed and control sample, by using a field gradient to resolve the two samples. A specific device for the temperature control was also built, to avoid misunderstandings in the evaluation of the rates of reaction.

2. Materials and methods

2.1. Materials and general methods

Potassium nitrosodisulfonate, $K_2NO(SO_3)_2$, or Fremy's salt was from Aldrich Chimica (Milano, Italy). Ascorbic acid was from Sigma (St. Louis, MO). Other chemical compounds, reagent-grade, were from Fluka (Buchs, Switzerland). All the solutions were made in bidistilled water.

The stock solution, 33 mM, of the inorganic nitroxide Fremy's salt was prepared by dissolving the appropriate amount of solid $K_2NO(SO_3)_2$ in a saturated solution of K_2CO_3 and was maintained, protected from air and light at 4 °C, for a maximum of 1 month. The ascorbic acid stock solution, 12 mM, was daily prepared by dissolving it in 0.1 M Na_1/Na_2-phosphate buffer (pH 7.5). Before each measurement, Fremy's salt and ascorbic acid were diluted in the above buffer as appropriate.

In EPR experiments, Fremy's salt was mixed with ascorbic acid to final concentrations of 17 and 1.2 mM, respectively (final pH=8.0). The ascorbic acid final concentration was chosen in order to optimize the rate of the studied reaction [12].

The mixed solution was immediately divided in two samples: one of them was positioned in the Helmholtz coils at steady or pulsed intensity and the other in environmental field (50 μT–0.5 G), at such a distance as the influence of Helmholtz coils field was negligible.

As sample tubes 50 μl quartz micropipettes (Corning, New York, USA), accurate to contain ±0.5% of the rated volume, were used.

From each pool of the solution, 10 or more capillaries were filled and the pairs were then used jointly for the parallel EPR measurements.

Since the total reaction of Fremy's salt disappearance is durable for a few hours, a pair of capillaries was taken for EPR measurement every 20 min, to sample the time course of the reaction. This was the protocol selected to measure the rate of a reaction necessarily carried out in the exposition apparatus, which is external to the measuring instrument. Each couple of capillary was detected several times, then substituted.

However, in this framework the EPR measurement was assumed to have a negligible effect at least on the first spectrum of each couple of samples, because of the shorter measurement time in the cavity. The EPR main field was thus used only as a reading field, and had a minor effect

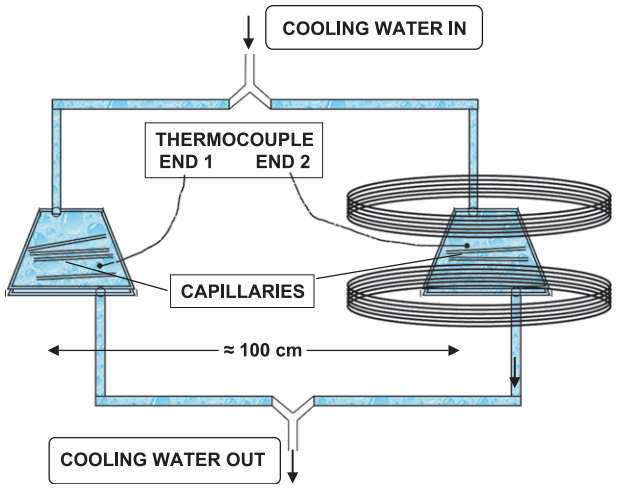

Fig. 1. Scheme of the exposure and temperature control systems.

with respect to the external magnetic fields in which the reaction was carried out.

The EPR X-band spectrometer, 9.5 GHz, was a conventional assembly of Bruker units (Bruker Spectrospin, Milano, Italy): a magnet B-M8, a resonant cavity 4108 TMH/9101, and a microwave bridge ER040XR, equipped with the field controller BH15.

The spectrometer operated at a central magnetic field near 348 mT (3480 G), scan range 0.8 mT (8 G), sweep time 35 s, time constant 100 ms, modulation frequency 100 kHz, modulation amplitude 100 μT (1 G). The effective 28 mW microwave power on the samples was generated by a 280-mW microwave power source (10 dB attenuation).

The temperature inside the resonant cavity, checked at the beginning and the end of each experimental session, was 18.0 °C with accuracy of ±0.5 °C.

The peak-to-peak amplitude of Fremy's salt EPR spectra was used to determine the level of this probe during the reaction with the scavenger. Only the central line of the Fremy's salt triplet was observed in the signals of the couple of samples, evaluated in parallel and resolved by the gradient.

2.2. Pulsed ELF-EMF and static exposure system

In the present study, samples were exposed to the Extremely Low Frequency-Electro Magnetic Field (ELF-EMF) produced by a pair of Helmholtz coils which generate, in the central region, a highly homogeneous field (see Fig. 1). The coils were supplied by a dedicated power pulse generator built in our laboratory, which produce an effective magnetic field in the range of 0–2.8 mT (0–28 G), shaped by a square wave, ranging from 1 to 75 Hz. The distribution and homogeneity of the field by Helmholtz coils was mapped and calculated using a Laplace equation simulation program that takes into account the coils finite dimension. Field distribution intensity was also measured with a digital teslameter (DYM-141: Group 3, Danfysin, Wellington, New Zealand) equipped with a Hall probe (LPT-141-125). The field region with homogeneity better than 1/1000 was large enough to restrain 10 capillaries.

In the exposition protocol, a square wave signal, 50% duty cycle, with the frequency of 50 Hz was used to give to the samples an effective magnetic field of 0.5, 1.0, 1.5 mT (5, 10, 15 G).

For stronger field expositions, static field of 4 and 6 mT (40 and 60 G) were used, generated by the same pair of Helmholtz coils, supplied by a APLAB preset regulated DC power supply L1635 (Pentatron Electronic Instrumentation, Venaria Reale, Torino, Italy), ranging in 0–16 V; 0–35 A.

2.3. Temperature control

Kinetic parameters, which are expected to reveal the effect of magnetic field exposure, are so sensitive to the temperature, that particular attention had to be paid in controlling the temperature of studied samples. Thus, the two sets of capillaries, stored inside and outside the magnetic field, were strictly maintained at the same temperature.

Joule effect in the coils might actually generate uncontrolled thermal increase in the exposed samples. To avoid this, a system was built made of two empty glass plates with a gap inside, feed by a continuous water flux. One plate was positioned inside the Helmholtz coils and the other far away. Each set of capillaries was laid down in thermal contact with the plates during exposition time. Refrigerating glass plates were at the temperature of the aqueduct, whose thermostatic properties are very reliable. The temperature of the contin-

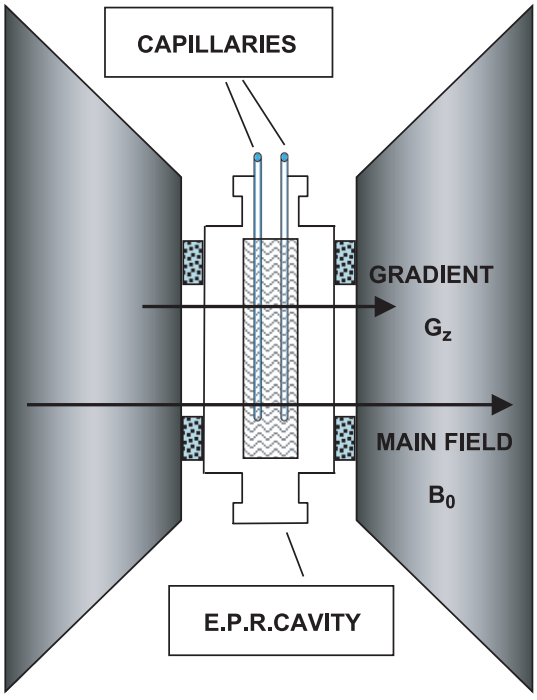

Fig. 2. View of the EPR cavity in the magnet gap. The directions of both the main and gradient fields are reported, as well as the position of two capillaries located side by side.

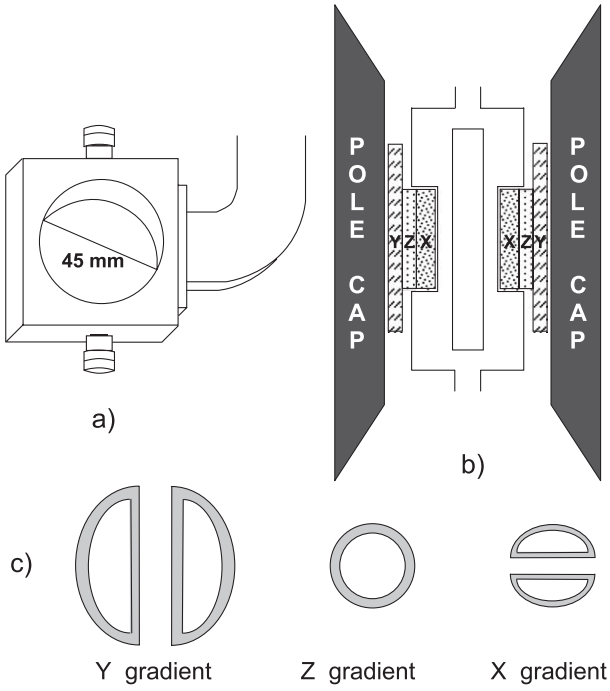

Fig. 3. A sketch of the cavity with the gradient device. Panel (a) the TM_{110} cavity showing the cylindrical region used to locate the gradient coils. Panel (b) cavity section view with the gradient coils inserted between the pole caps. Panel (c) detailed shape of the winding set assembled on each side of the cavity. The whole gradient coils assembly is done of the circular G_z and the D-shaped G_x and G_y coils.

uous water flux was 11.0±0.5 °C. The whole assembly is shown in Fig. 1.

The temperature stability in the capillary pair was checked by direct measurement in differential mode with the two thermocouple ends fixed on each plate (thermocouple sensitivity ≅ 50 μV/°C). In this way, it was possible to measure the difference of temperature (ΔT) between the two refrigerating plates with an accuracy corresponding to less than 0.05 °C. The ΔT in the pairs of samples was always maintained in the limit of less than 0.1 °C.

2.4. Gradient coils

A pair of samples, one stored inside and the other outside the Helmholtz coils, were inserted together in the cylindrical TM_{110} cavity. The two quartz capillary tubes are positioned, side by side, perpendicularly to the direction of the stationary main magnetic field (Fig. 2). Their signals were discriminated by applying an appropriate field gradient. By this approach, being the boundary conditions strictly homogeneous for each pair of capillaries, we obtained a sort of "parallel detection".

The whole spatial resolution of a sample in the cavity can be generally achieved using stationary field gradients along the three perpendicular axes. Taking the z-axis, as usual, along the direction of the main magnetic field, the three gradients are $G_x=\delta B_z/\delta x$, $G_y=\delta B_z/\delta y$ and $G_z=\delta B_z/\delta z$. These are usually obtained by Anderson gradient coils added to the pole caps of the magnet. In our case, the particular geometry of the cylindrical TM_{110} cavity (Fig. 3, panels a and b) consent to accommodate a part of the coil assembly in its central circular cutout.

A novel shape was thus proposed for the complete set of field gradients coils [13]. Pairs of D-shaped coils were designed for the gradients G_x and G_y perpendicular to the main field. The G_z component was instead generated by two parallel circular coils of reversed current, with radius equal to $1/\sqrt{3}$ of their distance. The entire G_x winding and a part of G_z are included in the inner cavity cutout. Fig. 3 shows the shape and arrangement of the gradients coils. The dimensions and the technical characteristics of the device are reported in Table 1.

The gradient coils are powered by a home built current regulated power supply controlled by an eight-bit ADC. The maximum current that can be used for each coil is of about 4 A, which corresponds to fields gradients G_x, G_y, G_z of 256, 296 and 552 mT/m, respectively. Computation of the field gradient was carried out using the Laplace equation, as for the Helmholtz coils.

3. Results and discussion

3.1. Radical pair reaction

Fremy's salt reduction induced by ascorbic acid was tracked by the EPR line intensity of the nitroxide signal. It proceeds in two steps: at first, there is a sudden loss and successively a slower decay with a rate independent on the

Table 1
Geometrical and electrical characteristics of the gradient coils

Gradient coils	Mean radius of the windings (mm)	Turns	Resistance[a] (Ω)	Inductance (mH)	Air core gradient[b] (coils applied on pole caps) (mT m^{-1} A^{-1})
X	21.0±0.1	37	1.56±0.01	656±1	64±2
Y	20.0±0.1	44	2.80±0.01	1176±1	74±2
Z	19.0±0.1	50	1.82±0.01	616±1	138±2

[a] Resistance was measured with a four-wire accuracy.
[b] Field gradients are reported for 700 mA current flowing in each pair of coils. Errors in determination of the field gradients are essentially due to the sensitivity of the Hall probe.

Fremy's salt concentration [12,14]. Although the family of ascorbic acid derived species is well known [15], a detailed description of the intermediate steps in this reaction is not found in the literature. A radical derived by ascorbic acid, most likely the ascorbyl radical $A^{-\cdot}$, is responsible for the formation of the pair with the Fremy's salt radical.

RPM determines the interaction within the pair, which accounts for the observed field effects about the reaction rate.

The formation and decay of ascorbate radical anion intermediate can be outlined by the following equations [11].

$$AH^- + (SO_3K)_2NO^{\cdot} \rightarrow A^{-\cdot} + (SO_3K)_2NO^- + H^+$$
(formation of ascorbyl) (1)

$$A^{-\cdot} + (SO_3K)_2NO^{\cdot} \rightarrow A + (SO_3K)_2NO^-$$
(RPM—decay of ascorbyl) (2)

Reaction (1) occurs without radical pair interaction and generates the ascorbyl radical, which in Eq. (2) reacts again with Fremy's salt in a true RPM. The overall process is complicated by the simultaneous decay of ascorbyl by reverse dismutation.

$$2A^{-\cdot} + H^+ \rightarrow AH^- + A \text{ (dismutation)}$$

This last process subtract a fraction of ascorbyl radical from RP interaction, and restores the reagent specie AH^- during reaction time, so that ascorbyl can be continuously reformed in solution from Eq. (1) [11]. As a consequence, the reducing agent appear to be very effective, although the stoichiometric ratio of the reagents is unfavourable: 1.2 mM ascorbic acid towards 17 mM Fremy's salt [12]. This feature might also be responsible for the long-lasting timing of the reaction.

During the reaction, the fraction of the Fremy's salt reduced by ascorbate (non-radical pair) is not influenced by magnetic field, by any reasonable mechanism. Fremy's salt and ascorbyl radical on the contrary are a radical pair, type F-pairs, which are reactive for 25% (i.e. only in the S state), in each following reencounters. This percentage is only altered by the phenomenon of interconversion between S and T states, which depends on the relative energy gaps that are clearly influenced by the value of the magnetic field.

3.2. Effects of magnetic fields on the nitroxide EPR signal decrease rate

EPR data shows the decrease of Fremy's salt central line during its reduction. The parallel detection using the G_z gradient allows to detect at the same time the signal of the two samples in the cavity (exposed/control). The simultaneous measurement avoids all the possibly deviation due to instrumental setting. The gradient easily resolves the two samples and the peak-to-peak amplitude of each signal is used to represent the parallel kinetic decay of the two samples. The temperature control allows the comparison between the timing of kinetic decay of exposed and control sample in the same conditions.

Immediately after the mixing of the radical with the reductant ascorbic acid, the two samples showed an identical peak amplitude, then, in the successive evaluation of pairs of capillaries with greater time of exposition, the amplitude differences between the capillary signals become more and more pronounced. A "parallel" signal from the final part of a kinetic decay, is reported in Fig. 4 in which the two amplitude are clearly differentiated.

The sample exposed to the external magnetic field in Helmholtz coils always showed a slower decrease with respect to the one rested in environmental field.

The magnetic field cause the increase by Zeeman effect of the gap in energy levels $T_{+1}$and T_{-1} and reduce the probability of S/T mixing [8]. As expected by this theory, the rate of Fremy's salt reduction is decreased by means of magnetic field exposition.

In Fig. 5, the whole kinetic of a couple exposed/control sample is shown, as an example. The amplitude values of the exposed sample in the final part of the kinetic becomes to be considerably lower than the control one and the zero level signal was earlier reached by the signal of the sample stored in environmental field. The couple of two samples, once inserted in the resonant cavity, whose temperature was higher than the cooled plates, jointly undergo an increase in magnetic field and in temperature, whose effects are in competition. The temperature effect is the more effective and produce a temporary increase in the reaction rate. This is visible in Fig. 5 on the slope of the data point, where each

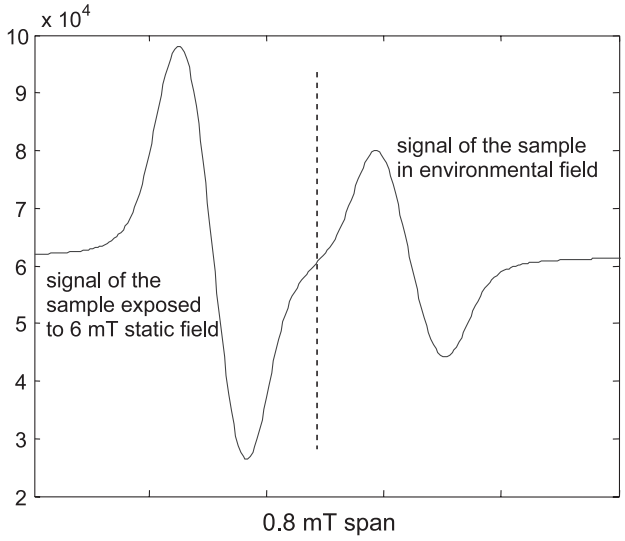

Fig. 4. Parallel detection of the Fremy's salt triplet central line upon interaction with ascorbic acid, using a G_z gradient of 7×10^{-2} T m^{-1}. The figure shows the signal of a pair of exposed and control sample, recorded 192 min after the mixing. Instrumental settings are reported in the experimental section. The gradient allows to separate the contributions of the pair of capillaries in the cavity, which for clarity are shown separated by a dotted line.

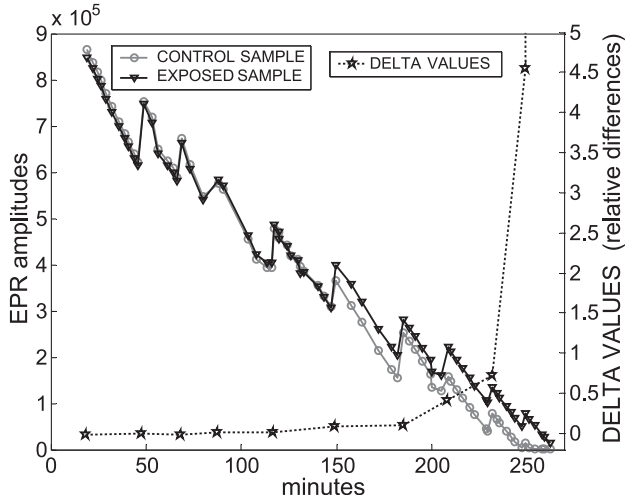

Fig. 5. Solid lines show the time course of Fremy's salt signal decay of the sample stored in environmental field (control) and the sample exposed to 6 mT static magnetic field (exposed). Multiple measurements were performed for each pair during its stay in the cavity. Dotted line indicates the relative differences in amplitude between exposed/control samples. Delta values Δ (defined in the text) refers only to the first measurement of each pair of capillaries and their position indicates as well each first spectrum after replacing the couple of capillaries in the cavity.

jump in the values indicate the substitution of the capillary pair in the cavity.

By using our parallel detection, it is helpful to express the relative difference of amplitudes as $\Delta=(A_{EXP}-A_{ENV})/A_{ENV}$, where A_{EXP} and A_{ENV} are the peak-to-peak amplitude of exposed and control sample signals. This choice has the effect to enhance the differences in the final part of the kinetic decay, as is evident in Fig. 5. A Δ value of 1 means that the not exposed signal is double with respect to the exposed one.

For the Δ calculation, reported as dotted line in the same figure, only the amplitudes of the first value, measured immediately after replacement of the pair of capillaries, were used. These data, due to their shorter stay in a different environment, are less influenced by the measuring system. In fact, their time scan (35 s) is negligible with respect to the total observed time-course of field exposure (250–300 min).

The final parts of kinetic decay are clearly demonstrated to be function of the applied magnetic field.

The same behaviour of the curves were observed also in the case of static (4 mT) or pulsed (0.5, 1.0 and 1.5 mT) magnetic field indicating that the imposed magnetic field affects the reagent pair interaction decreasing the rate of radical disappearance (data not shown).

This behaviour might be ascribed to RPM and the found Fremy's salt kinetic rate decrease seems to be in line with this theory.

This RPM effect, studied up to now in spectrophotometry, is easily detectable with the present technique, because the EPR evaluation does not need chromogenic reagents and/or products. Another advantage of this approach is the possibility to detect and to follow both the radicals and/or intermediates involved in the reaction, as in our case the ascorbyl radical ($A^{-\cdot}$) [11].

Furthermore, the EPR technique might offer the possibility to utilize the main magnetic field to induce and to detect, at same time, the effects on radical pair reactions.

4. Conclusions

In this work, we developed a technical device which allows the direct evaluation, through EPR spectroscopy, of weak static and pulsed magnetic field strength effects on biological radical reactions rate, as in the present study the Fremy's salt–ascorbic acid radical pair. This kind of "double beam" EPR spectroscopy is based on the resolution of the signal by addition of a stationary field gradient to discriminate between exposed and control sample, present at the same time in the EPR cavity. The temperature homogeneity of the couples is obtained by a refrigerating system controlled by a thermocouple. The strictly controlled boundary conditions and the parallel detection of the samples allow the determination of the small effect caused by magnetic fields.

This device as well as the EPR spectroscopy are useful tools for studies related with the radical pair mechanism (RPM) and magnetic field effects, although further experiments are necessary for a more quantitative analysis of this effect.

Consequently, this method will be applicable in the analysis of radical pairs either in solution or in living cells in suspension and allows kinetic studies also in the presence of not chromogenic substrates and/or products.

References

[1] M.F. Barnothy, Biological Effects of Magnetic Fields, Plenum Press, New York, USA, 1964.
[2] B. Brocklehurst, K.A. McLauchlan, Free radical mechanism for the effects of environmental magnetic field on biological systems, Int. J. Radiat. Biol. 69 (1996) 3–24.
[3] C.B. Grissom, Magnetic field effects in biology: a survey of possible mechanisms with emphasis on radical-pair recombination, Chem. Rev. 95 (1995) 3–24.
[4] J.C. Scaiano, F.L. Cozens, N. Mothat, Influence of combined AC–DC magnetic fields on free radicals in organized and biological systems. Development of a model and application of the radical pair mechanism to radicals in micelles, Photochem. Photobiol. 62 (1995) 818–829.
[5] J.C. Scaiano, N. Mothat, F.L. Cozens, J. McLean, A. Thasandote, Application of the radical pair mechanism to free radicals in organized systems: can the effects of 60 Hz predicted from studies under static fields? Bioelectromagnetics 15 (1994) 549–554.
[6] J.R. Woodward, R.J. Jackson, C.R. Timmel, P.J. Hore, K.A. McLauchlan, Resonant radiofrequency magnetic field effects on a chemical reaction, Chem. Phys. Lett. 272 (1997) 376–382.
[7] H. Hayashi, Magnetic field and magnetic isotope effects on reactions of radical pairs, in: S. Nagakura, H. Hayashi, T. Azumi (Eds.), Kodansha, Tokyo, Japan, 1998, pp. 7–47.

[8] H. Hayashi, Introduction to Dynamic Spin Chemistry: Magnetic Field Effect Upon Chemical and Biochemical Reactions, World Scientific Publisher, Singapore, 2004, pp. 21–30.
[9] S.N. Batchelor, C.W.M. Kay, K.A. Mc Lauchlan, I.A. Shkrob, Time-resolved and modulation methods in the study of the effects of magnetic fields on the yields of free radical reactions, J. Phys. Chem. 97 (1993) 13250–13258.
[10] S.N. Batchelor, K.A. Mc Lauchlan, I.A. Shkrob, Reaction yield detected magnetic resonance in exciplex systems: II. Time resolved and pulse studies, Mol. Phys. 75 (1992) 521–561.
[11] G. Gualtieri, S. Colacicchi, C. Corvaja, First observation of very long-lasting emissive polarization in EPR spectra of Fremy's salt when reduced by ascorbic acid. An unusual aspect of the radical pair mechanism, J. Chem. Soc., Perkin Trans. 2 11 (2002) 1917–1921.
[12] S. Colacicchi, V. Carnicelli, G. Gualtieri, A. Di Giulio, EPR study of Fremy's salt nitroxide reduction by ascorbic acid: influence of the bulk pH values, Res. Chem. Intermed. 26 (2000) 885–896.
[13] G. Gualtieri, G. Placidi, A. Sotgiu, S. Del Monaco, Integration of D-shaped gradient coils in a Bruker TM circular cavity for X-band electron spin resonance imaging, Rev. Sci. Instrum. 66 (1995) 3715–3716.
[14] A. Di Giulio, V. Carnicelli, S. Colacicchi, G. Gualtieri, An EPR study of lipid vesicle as contrast agent vectors, Appl. Magn. Reson. 13 (1997) 553–559.
[15] D. Njus, V. Jakular, J. Zu, P.M. Kelley, Concerted proton–electron transfer between ascorbic acid and cytochrome B561, Am. J. Clin. Nutr. 54 (1991) 79S–83S.