

Seventh Meeting of the Benelux EPR Society

Program Book of Abstracts List of Participants



April 27, 1999
University of Leiden
Huygens Laboratory
Niels Bohrweg 2
Leiden - The Netherlands



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Organizing Committee:
Peter Gast
Edgar Groenen



Scientific program

- 09.00-10.00 Registration + coffee
10.00-10.05 Welcome By Prof. Schoemaker, Chairman
- 10.05-10.55 **Invited lecture by K.A. McLauchlan**
The dynamics of radical pairs as studied through CIDEP
- 11.00-11.30 Coffee break
11.30-11.50 M. Huber, Univ. Leiden
On the electronic structure of radicals in the primary process of photosynthesis.
- 11.55-12.15 S.V. Nistor, Univ. Antwerp
ESR of Cu^{2+} ions in photorefractive $\text{Bi}_{12}\text{GeO}_{20}$ crystals
- 12.20-12.40 M. Hoebeke, Univ. Luik
Electron spin resonance study of bacteriochlorin a incorporated into membrane models.
- 12.45-14.10 Lunch
14.15-14.35 A. Priem, Univ. Nijmegen
High spin systems made easy with high frequency EPR
- 14.40-15.00 M. Stefan, Univ. Antwerp
ESR probing with T1^0 centers of ferroelectric phases in Rb_2ZnCl_4 crystals
- 15.05-15.25 M v. Gastel, Univ. Leiden.
Axial ligation in blue-copper proteins
- 15.30-15.55 Tea break
16.00-16.20 P.J.M. van Bentum, Univ. Nijmegen
The Nijmegen high frequency EPR facility: technical developments and application to colossal magnetoresistance materials.
- 16.25-16.45 I. Proskuryakov, Univ. Leiden
Structural information on photosynthetic RCs obtained from magnetophotoselection measurements of spin-polarized EPR signals
- 16.50 General meeting of Benelux EPR Society
17.15 Reception

The dynamics of radical pairs as studied through CIDEP

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Whenever transient free radicals are observed within the first microsecond or so of their creation they exhibit a spin disequilibrium said to be due to Chemically Induced Dynamic Polarization (CIDEP). In photochemically-generated radicals this often has two independent origins, the Triplet Mechanism (TM) and the Radical Pair Mechanism (RPM). The TM is present in the ensemble of radicals at the instant of its creation, but the RPM arises in that sub-ensemble of radicals which, having been created together, subsequently re-encounter in the geminate period of the radical combination process. In principle the ratio of the two contributions to the observed spin polarization yields a direct measure of the fraction of radicals produced which do re-encounter. This is seminal to all radical pair processes, and to the whole of radical combination chemistry, including photochemistry.

But the very same interactions which produce the RPM polarization also render a pair created in the triplet state able to react at a later time. The consequence is that the results yield the fraction of radicals that survive reaction in the geminate cage. The reaction probability then can be studied as a function of the viscosity of the solution, information relevant to the effects of magnetic fields on radical reactions.

On the Electronic Structure of Radicals in the Primary Processes of Photosynthesis

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The origin of the high efficiency of energy conversion in the primary processes of photosynthesis is not fully known. Thus, understanding the mechanism of the underlying coupled energy and electron transfer processes is an important task. A number of EPR techniques has been used to investigate the electronic structure of the cofactors involved, which is considered as one of the factors entering the electron transfer efficiency. The talk will focus on the role of the primary electron donor (D), a dimer of Bacteriochlorophyll molecules in bacterial photosynthesis (reviewed in [1]). The electronic structure of the cation radical of D ($D^{\bullet+}$) is determined by ENDOR (Electron Nuclear Double Resonance) and mutants [2] are used in order to determine how it is affected by amino acid residues in the protein environment. Combined with the results from other spectroscopic and structural methods a detailed picture of the fine-tuning of the chromophore by the environment can be derived. Comparison with the results of ENDOR experiments on the triplet state of D (D^T) [3] give an indication about the distribution of different orbitals, in particular about those involved in charge separation.

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ESR OF Cu^{2+} IONS IN PHOTOREFRACTIVE $\text{Bi}_{12}\text{GeO}_{20}$ CRYSTALS

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Copper doped, cubic $\text{Bi}_{12}\text{GeO}_{20}$ (BGO) crystals with sillenite structure are known to exhibit photorefractive properties [1]. Except for a short, erroneous paper [2] dedicated mainly to ESR on polycrystalline $\text{BGO}:\text{Cu}^{2+}$, there seem to be no other published Electron Spin Resonance (ESR) studies on this crystal-paramagnetic impurity system.

We present here the results of a systematic ESR (X- and W- band) study on BGO single crystals co-doped in the melt with copper and vanadium. The angular dependence of the ESR lines has been determined in both (110) and (100) planes, from room temperature (RT) down to 3.8K. All observed lines were attributed to Cu^{2+} ions. Vanadium seems only to favour the insertion of copper into the crystal lattice.

The X-band ESR measurements revealed the presence of a broad ($\Delta H \sim 50\text{mT}$) structureless line, slightly anisotropic in both shape and position, extending in the effective g-values range from about 2.0 to 2.4. Except for the line intensity increase, no other significant changes have been observed by decreasing the measuring temperature.

Computer line shape simulations of the observed spectra suggested the presence of at least one type of Cu^{2+} center with axial symmetry around one of the $\langle 111 \rangle$ axes, with $g_{\parallel} = 2.28$, $g_{\perp} = 2.04$ and 20mT linewidth.

As expected from the X-band lineshape analysis, the ESR transitions due to Cu^{2+} centers at lattice sites with different orientation of the local axes are fully resolved in the W-band spectra. Even the hyperfine structure from the copper nuclei with nuclear spin $I=3/2$ could be observed for certain magnetic field orientations.

The analysis of the resulting spectra resulted in the identification of two types of axial Cu^{2+} centers with different spin Hamiltonian parameters and orientation of the main axes. The resulting spin Hamiltonian parameters are discussed in the frame of the crystal field theory. The present study is a good example of the capabilities of the W-band ESR in resolving spectra which, at usual microwave frequency bands, overlap beyond recognition.

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Electron spin resonance study of Bacteriochlorin *a* incorporation into membrane models.

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Bacteriochlorin *a* (BCA) is attracting considerable attention in view of its potential use in light-induced process as photodynamic therapy (PDT) which is an innovative modality for the treatment of small and superficial tumours. Indeed, particular attention is devoted to sensitizers presenting a tumor cells preferential retention and absorbing above 600 nm. In this spectral region, endogeneous tissular components are transparent to the incident radiation, minimizing the risk of photodamage at the level of cells or tissues not containing the photosensitizers. Moreover, under light irradiation, these dyes must produce active oxygen species able to react with the surrounding biological components inducing lethal damages. BCA answers completely to these preliminary requirements.

Our previous results concerning the BCA photodynamic activity are consistent with a mixed Type 1 / Type 2 reaction process. However, it is very complicated to draw conclusions on the exact mechanism which induces tumour necrosis since during PDT, the reaction pathway strongly depends on the oxygenation within the tumour, the singlet oxygen (¹O₂) lifetime in a particular environment and the stability of generated radicals. Type 1 reaction processes are expected to be favoured in polar media while, in contrast, Type 2 mechanisms predominate in hydrophobic environments. So, the antitumoral activity of a sensitizer appears to be mainly governed by its ability to locate or not in the membrane. Its primary localization in biomolecules strongly depends on its lipophilic or hydrophobic character.

In this work, the distribution properties of BCA inside the liposomes suspension was studied using a spin label occupying the aqueous phase and a series of doxyl stearic acids with the nitroxyl radical at different depths in the lipid membrane. The more efficient quenching of 5-DSA together with the absence of any significant reduction for 12-DSA and 16-DSA are in favour of a localisation of the dye near the polar head interface. Moreover, the observed reduction of the water soluble carbamoyl clearly indicates that at least a portion of BCA molecules resides close to the membrane surface.

High Spin Systems made easy with High Frequency EPR

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The EPR spectra of high spin systems are of considerable interest in inorganic and bioinorganic chemistry. The quantitative analysis of such EPR spectra is accompanied by a number of experimental and theoretical difficulties. The most obvious problem encountered is the often relatively large zero field splitting between the Kramer's doublets ($M_s=1/2, 3/2, 5/2 \dots$). For instance, this has limited nearly all studies on biological high spin Fe(III) to only one (or two) intra-doublet transition.

With the recent development of high frequency EPR spectrometers it is now possible to observe the entire spectrum, resulting in a far more accurate determination of the spin Hamiltonian parameters, in particular the zero field splitting parameters.

By doing a multi-frequency analysis of high-spin model systems with a relatively small zero field splitting, we develop a methodology which is also applicable for (biological) systems with high zero field splittings. Also, the correlation between zero field parameters and local structure is investigated.

ESR PROBING WITH Tl^0 CENTRES OF FERROELECTRIC PHASES IN Rb_2ZnCl_4 CRYSTALS

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Rb_2ZnCl_4 crystals exhibit successive structural phase transitions (SPT) from a normal orthorhombic ($Pm\bar{c}n$) paraelectric phase to an incommensurate phase at $T_1 = 303K$, followed by a transition to a commensurate orthorhombic ($P2_1cn$) ferroelectric phase at $T_{C1} = 192K$ and to a monoclinic ($C1c1$) ferroelectric one at $T_{C2} = 74K$.

In this work we are investigating the local symmetry changes induced by the low temperature phase transition $P2_1cn - C1c1$, using Tl^0 atom centres as paramagnetic probes. The measured Rb_2ZnCl_4 crystals, doped in the melt with 0.2mol% $TlCl$, were x-ray irradiated at 80K. ESR measurements in the X-band were carried out on a wide temperature range, from 5 to 230K. Pulse annealing experiments were also performed in order to determine the production properties of the centres.

Anisotropic spectra attributed to several Tl^0 -type centres could be observed up to 200K. In the orthorhombic ferroelectric phase four different centres were observed. Three of them, with very similar spin-Hamiltonian parameters, seem to have the same original basic structure, slightly distorted by the tripling of the unit cell due to the incommensurate-commensurate SPT. The fourth centre, with broader lines, has an entirely different structure, reflected in the different values of the spin - Hamiltonian parameters and isotropic hf contribution [1] ($A_g \sim -1$ mT, compared to the average 60 mT calculated for the other three centres).

The pulse annealing investigations showed no difference in the production properties of the four centres. They seem to be formed by electron trapping at a precursor Tl^+ centre, and their disappearance is related to the V_k [2] and Tl^{2+} [3] centres decay. No vacancies movement seems to affect their behaviour at higher temperatures.

By cooling the crystals below T_{C2} continuous changes in the ESR lines positions and number were observed. These changes are attributed to symmetry lowering effects and temperature induced hyperfine splitting variation, and they support the use of Tl^0 centres as new, sensitive paramagnetic probes for investigating SPT in these crystals.

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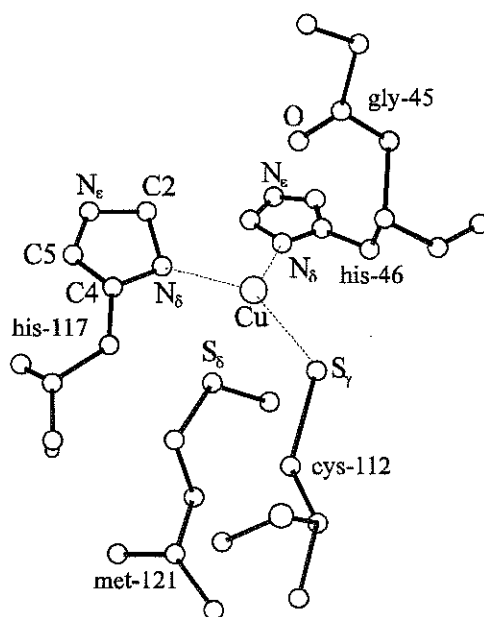
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Axial ligation in blue-copper proteins. A W-band EPR study of the azurin mutants M121Q and M121H

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Blue-copper proteins have been extensively studied because of their mediating role in electron-transfer processes. In the oxidised form, these metallo-proteins are characterised by an intense absorption around 630 nm and an unusually small copper-hyperfine interaction in the EPR spectrum. Both observations are related to a remarkable copper coordination which is highly conserved. The copper is ligated equatorially by the nitrogens of two histidines and the sulphur of a cysteine. The axial ligand position is usually taken by the sulphur of a methionin. This is not the case for stellacyanin where the axial position is taken by the oxygen of a glutamine and the EPR spectrum is rhombically distorted.

To investigate the role of the axial ligand as regards the wavefunction of the unpaired electron, we have performed high-frequency (95 GHz) ESE detected EPR studies on single crystals of two mutants of azurin in which the axial methionin has been replaced by either a glutamine (M121Q) or a histidine (M121H). According to X-ray, the replacement of the methionin by a stronger axial ligand pulls the copper out of the equatorial plane towards this axial ligand. It will be shown that for both mutants two distinct conformations of the copper site exist in the crystal. From the EPR studies, we have been able to determine the complete g -tensor for each conformation of the copper site and fixed the orientation of the principal axes with respect to the average copper site. The directions of the g_z axes reveal information about the electronic structure of the copper site for each mutant. The wavefunction of the unpaired electron will be discussed in relation to the out-of-plane displacement of copper and the orientation of the ligands with respect to copper.



The copper site of azurin

The Nijmegen high frequency EPR facility; Technical developments and application to colossal magnetoresistance materials

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The purpose of the UMBELLA project is to develop a multi-frequency EPR user facility for frequencies up to 1 THz and magnetic fields up to 30 Tesla. At present, the high field magnet laboratory in Nijmegen can offer EPR for external users at frequencies up to 300 GHz with a variety of sources and cavity configurations. Support from the EC large facility program and FOM-IGM makes this facility available to Dutch and EC scientists at no costs.

In this contribution we will present the technical details of the available configurations. Some examples are given to demonstrate the versatility of the system. In particular we will present multifrequency results on single crystal CMR materials $\text{La}_{1-x}\text{Ca}_x\text{MnO}_3$.

Magnetophotoselection study of radical pairs and triplet states in photosynthetic reaction centers

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The technique of magnetophotoselection utilising time-resolved direct detection EPR (DDEPR) is developed and applied to the study of spin-polarised triplet and radical pair (RP) states in photosynthetic reaction centers (RCs). The analysis of the spectra with magnetophotoselection provides information on the orientation of the optical transition dipole of the absorbing molecule and the dipolar axis of the RP relative to the magnetic axes of the triplet state or of one of the radicals from the RP. This data provides information on the structural organisation of the cofactors in RCs in solution. DDEPR is not very sensitive to spin-lattice relaxation, and allows studies of spin-polarised spectra in a wide temperature range.

DDEPR measurements were performed as described earlier [1]. The excitation laser light was first depolarised in a lightguide, and then repolarised with a Glan-Thompson prism. Each experiment comprised measurements with excitation light polarisation plane parallel and perpendicular to the magnetic field. Light intensity was kept low enough to prevent saturation of photoselection (usually ca. 0.1 mJ per 4-ns pulse). Excitation into the Q_y absorption band of the primary donor P was utilised (860-915 nm). Reaction centers of *Rb. sphaeroides* R-26 with the primary acceptor Q_A chemically reduced or removed were studied.

Simulation of the 3P spectra provide the values of the spherical co-ordinates of Q_y in the triplet axes frame ranging from $\theta=80^\circ$, $\phi=70^\circ$ to $\theta=90^\circ$, $\phi=90^\circ$, depending on the excitation wavelength. It is noteworthy that simulation of two independently measured spectra within the same set of fitting parameters considerably increases the precision of the angle determination, which in the given case is $\pm 5^\circ$. The ZFS parameters of 3P also change with λ_{exc} , which reflects the presence of primary donor subpopulations with varying optical and magnetic properties. Spectral shape of 3P in RCs with reduced Q_A changes in the temperature range 10 - 30 K. We attribute such behaviour to the relaxation effect of non-heme iron ion. If iron is replaced by non-paramagnetic zinc, or Q_A molecule is removed, this temperature dependence disappears. Recent data on photoselection of $^3P_{680}$, the primary donor in plant photosystem II will also be presented.

Spin-polarised EPR spectra of the radical pair state $P^+Q_A^-$ also demonstrate strong magnetophotoselection [2]. We will report on improved measurements and simulations of these signals.

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