

# Chapter 1

## Introduction

### 1.1 Electron Paramagnetic Resonance

Electron paramagnetic resonance (EPR) spectroscopy is one of the major sources of structural and dynamic information about paramagnetic species [1–4]. The concomitant development of molecular biology and pulsed electron paramagnetic resonance (EPR) spectroscopy during the last decades have increased the use of EPR spectroscopy to study materials as well as biological systems.

The progression of EPR spectroscopy can be easily followed on the basis of literature references concerning the keywords "EPR" and "pulsed EPR" (figure 1.1 gives an overview).

Two periods can be distinguished in the field of EPR. A dramatic increase in the number of publications/year between 1960 and 1970 and more continuous increase since 1980. In this period EPR became a standard method for the investigation of paramagnetic species because more and more commercial spectrometers became available.

Whereas EPR spectroscopy was dominated in the past by continuous wave (cw) methods, cw methods in NMR spectroscopy have now completely been replaced by pulsed techniques [5, 6]. Although the first magnetic resonance experiment performed in 1945 was an EPR experiment [7], it took almost three decades until pulsed EPR spectroscopy was widely accepted as an additional source of structural information in biochemistry, chemistry and material science.

After the first observation of an electron spin echo in 1959 [8] and one year later of several FIDs of organic radicals at X-band frequencies [9], the first observation of the electron spin echo envelope modulation (ESEEM) effect in 1961 [10] had far-

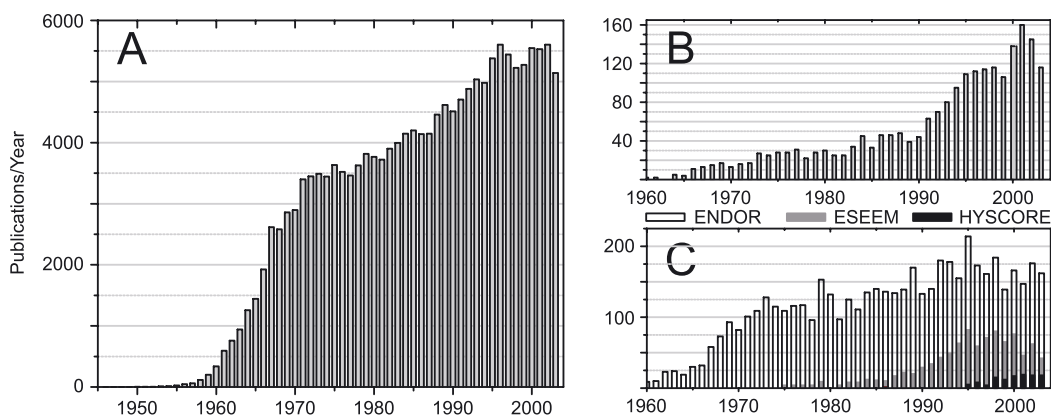


Figure 1.1: Publications concerning EPR spectroscopy. The literature search was performed on Februar 17<sup>th</sup>, 2004. A: Keywords: "EPR and ESR" after 1945, B: "Pulsed EPR" or "Pulsed ESR", C: "Electron nuclear double resonance" or ENDOR (no differentiation concerning pulsed or continuous wave methods), "Electron spin echo envelope" or ESEEM and HYSCORE

reaching consequences in developing new pulsed techniques for EPR experiments.

Today double-resonance experiments introduced by Mims in 1965 [11] as well as two-dimensional experiments introduced by Merks and de Beer in 1979 [12] and Freed in 1986 [13] belong to the standard repertoire of an EPR spectroscopist. With the availability of superconducting magnets, high-field high-frequency EPR spectroscopy increasingly becomes a routine method to study paramagnetic species [14, 15].

Pulsed EPR methods have been used successfully to investigate the local structure and dynamics of paramagnetic centers in biological samples [16]. In proteins such paramagnetic centres can be naturally stable cofactors, transiently generated radicals within a reaction cycle or artificial nitroxide spin lables attached to the enzyme. In contrast to NMR spectroscopy EPR is not restricted by the size of the system under study because only the paramagnetic centres and their interaction with the environment are spectroscopically visible.

## 1.2 Bioenergetics

The mitochondrial electron/proton transport, which is called the mitochondrial respiration, is one of the central processes in metabolism of eukaryotes. The electron transduction is coupled to the concomitant transport of protons through

the inner mitochondrial membrane generating the proton motive force, which is used by the ATP synthase to synthesize ATP from ADP and phosphate. In fact, several energy consuming processes in bacteria and chloroplasts are driven by proton gradients. This fundamental principle of energy conversion is the so-called chemiosmotic theory and was first introduced by Peter Mitchell [17].

In oxidative phosphorylation, the synthesis of ATP is coupled to the flow of electrons via a chain of electron-transferring enzyme complexes driven by a proton gradient across the inner mitochondrial membrane. Since oxidative phosphorylation exemplifies a fundamental theme of bioenergetics – the transmission of free energy by proton gradients – it is of great interest to study the principles of the electron flow through the mitochondrial membrane in greater detail.

### 1.3 Aim of This Work

The aim of this work was to study the coordination sphere of the iron-sulphur cluster N2 in NADH:Ubiquinone oxidoreductase (complex I) the first enzyme complex of the mitochondrial respiratory chain by ESEEM spectroscopy. Due to the overlap of the EPR signal of cluster N2 with the EPR signal of cluster N1, a new method for separation of overlapping EPR signals had to be developed (REFINE). The application of this method is first shown on the basis of simple model compounds and afterwards applied to the biological system complex I. The efficiency of the method as well as the new structural information derived for cluster N1 and N2 are discussed.

Finally a concept that allows the separation of more than two species is introduced. The method is an extension to REFINE and the applicability is demonstrated by numerical simulations and first examples are given.

### 1.4 Outline

This work is divided into several sections.

- Chapter 2 will give an overview about the respiration in different organisms. The electron transfer chain, protein complexes involved in the electron translocation through membrane as well as the structure and function of the individual complexes will be introduced.

- Chapter 3 will give an overview about the theory of EPR. An introduction to the density matrix formalism will be given and the standard pulsed experiments used in pulsed EPR are manifested on the basis of this theory.
- Chapter 4 gives an overview about the materials and methods used in this thesis. The principles of spectral analysis as well as the methodology of simulating EPR experiments are shown.
- In chapter 5 Relaxation Filtered Hyperfine Spectroscopy (REFINE) is introduced in order to separate the contribution of the two spectrally overlapping iron-sulphur clusters N1 and N2 of complex I. This chapter has already been published in *Biochemistry*.
- Chapter 6 gives a more detailed description about the filter efficiency of REFINE. The method is discussed with regard to commonly used EPR techniques. This chapter has already been published in *Journal of Magnetic Resonance*.
- Chapter 7 shows how pulsed EPR spectroscopy can be used to make contributions to the controverse discussion of the structural environment of the iron-sulphur clusters in complex I. The two iron-sulphur clusters N1 and N2 are investigated by pulsed hyperfine techniques and the results are discussed with respect to the structural environment.
- In chapter 8 a concept is discussed how the relaxation properties of individual paramagnetic species can be used to separate the contributions of more than two species. The concept is discussed numerically and a first experimental example is given.

The work finishes with a summary, the outlook, an appendix, a german summary and a curriculum vitae.

## 1.5 Publications

Parts of this work have already been published or presented at conferences.

### Articles

- Th. Maly and T. F. Prisner, Relaxation Filtered Hyperfine Spectroscopy (REFINE), *Journal of Magnetic Resonance*, 2004, 170 (1), 88 – 96
- Th. Maly, F. MacMillan, K. Zwicker, N. Kashani-Poor, U. Brandt and T. F. Prisner, Relaxation filtered hyperfine (REFINE) spectroscopy: A novel tool for studying overlapping biological EPR signals applied to mitochondrial Complex I, *Biochemistry*, 2004, 43 (13), 3969-3978
- Th. Maly, F. MacMillan, K. Zwicker, N. Kashani-Poor, L. Grgic, U. Brandt and T. F. Prisner, Complex I studied by pulsed EPR Spectroscopy, *Biochim. Biophys. Acta*, EBEC Short Reports, 2002, 12, 191
- P. Faller, Th. Maly, A. W. Rutherford and F. MacMillan, Chlorophyll and Carotenoid Radicals in Photosystem II Studied by Pulsed ENDOR, *Biochemistry*, 2001, 40 (2), 320-326
- Th. Maly et al., Cluster N1 of Complex I from *Yarrowia Lipolytica* Studied by Pulsed EPR Spectroscopy, 2004, Manuscript in preparation

### Posters<sup>1</sup>

- **Th. Maly**, F. MacMillan and T. F. Prisner, Overlapping EPR Signals: T<sub>1</sub>-Selective Pulsed Experiments, 33<sup>rd</sup> Annual International Meeting ESR Group RSC, Norwich, 2000
- **Th. Maly**, F. MacMillan and T. F. Prisner, Overlapping EPR Signals: T<sub>1</sub>-Selective Pulsed Experiments, DFG-Rundgespräch, Hirschegg, 2000
- **Th. Maly**, K. Zwicker, N. Kashani-Poor, F. MacMillan, U. Brandt, T. F. Prisner, Direct Evidence for the nitrogen ligation of the N2-FeS-Cluster in complex I from *Yarrowia Lipolytica*, 10<sup>th</sup> International Conference on Bioinorganic Chemistry (ICBIC), Florence, 2001

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<sup>1</sup>The presenting author is given in bold.

- **Th. Maly**, K. Zwicker, L. Grgic, F. MacMillan, U. Brandt, T. F. Prisner, The Iron-Sulphur Clusters N1 and N2 of Complex I of *Y. Lipolytica* Studied by Pulsed EPR, 6<sup>th</sup> European Conference on Bioinorganic Chemistry (EUROBIC), Lund, 2002
- Th. Maly, **F. MacMillan**, K. Zwicker, N. Kashani-Poor, L. Grgic, U. Brandt, T. F. Prisner, Complex I studied by pulsed EPR Spectroscopy, European Bioenergetics Conference (EBEC), Arcachon, 2002
- **Th. Maly**, F. MacMillan and T. F. Prisner, Relaxation Filtered Hyperfine (REFINE) Spectroscopy, GdCh Fachgruppentagung, Leipzig, 2003

# Chapter 2

## Mitochondrial Respiration

This chapter introduces the respiratory chain on the basis of different organisms and provides an overview of the structure and function of the enzyme complexes involved in electron and proton translocation. The electron transfer pathway through the different complexes will be discussed followed by a more detailed discussion of the state-of-the-art model of the structure of complex I introducing the controversial discussion about the location of the active sites of complex I. Since parts of this work carry out a structural characterisation of two iron-sulphur clusters in complex I by EPR spectroscopy, the last part of the introduction gives a short overview of different types of iron-sulphur clusters in proteins, their electronic structure and characterisation using EPR spectroscopy. Some parts are taken from textbooks [18–20] and only specific parts are labelled with individual citations according to the original literature.

### 2.1 The Respiratory Chain

One of the central processes in energy metabolism of higher plants, eukaryotes and bacteria is the electron transport in the membrane. In mitochondria this process is called mitochondrial respiration. This electron translocation is linked to a vectorial pumping of protons across the inner mitochondrial membrane generating the so-called proton motive force, which is used by complex V – the ATP synthase to synthesize ATP from ADP and phosphate. This fundamental principle of energy conversion is the so-called chemiosmotic theory and was first introduced by Peter Mitchel [17]. In oxidative phosphorylation, the synthesis of ATP is driven by the flow of electrons from NADH (or FADH<sub>2</sub>) to O<sub>2</sub> generating