Practical course:
Mössbauer effect on iron containing proteins

1. Abstract

Within this practical course the application of a nuclear physics method on a biological problem is demonstrated. A biological sample, namely the photo synthetic reaction center of the bacterium *Rhodopseudomonas viridis* is examined. For the determination of the 3 dimensional structure of this protein complex by X-ray crystallography, Deisenhofer, Huber and Michel got the Nobel prize for chemistry in 1988. But from this structural information alone one still cannot deduce the functional mechanisms of photosynthesis. Numerous other experiments are still necessary.
Within this practical course, the states of the different iron atoms within the reaction center which are essential for the photosynthesis are investigated with Mössbauer spectroscopy.

2. Biological basics

2.1 Photosynthesis

Nearly all energy which is used in biological systems, in particular the food of all animals, at last originates from the sunlight. The green plants absorb light and transform it by a multiway, complex process, the photosynthesis, into chemical energy. Oxygen is produced by the fission of water, which is essential for numerous organism.

$$6\ H_2O + 6\ CO_2 \longrightarrow C_6H_{12}O_6 + 6\ O_2$$

The energy necessary for this reaction is delivered by two quanta of light absorbed by two different protein complexes, the photosystem I (PS I) and the photosystem II (PS II). These and two additional protein complexes are embedded in the membrane of the chloroplasts, the organelles responsible for photosynthesis. The organelles are compartiments within the cell which are encased by membranes. At these four protein complexes, the so called "light reaction" happens (see Fig.1). At first the water is split at the Manganese cluster (Mn4) of PS II. The electrons which are set free in this process arrive at a pair of chlorophylls called "special pair P680". This can absorb light by what the electrons are energetically excited. The maximum of absorption is at 680 nm. Via a pheophytin (Ph) and the plastochinon Q_A the electrons reach the plasochinon Q_B. The P680, Ph, Q_A and Q_B are called prosthetic groups - these are components of proteins which are not build of amino acids. Q_B can pick up two electrons to become Q_B^{2-} . Q_B^{2-} picks

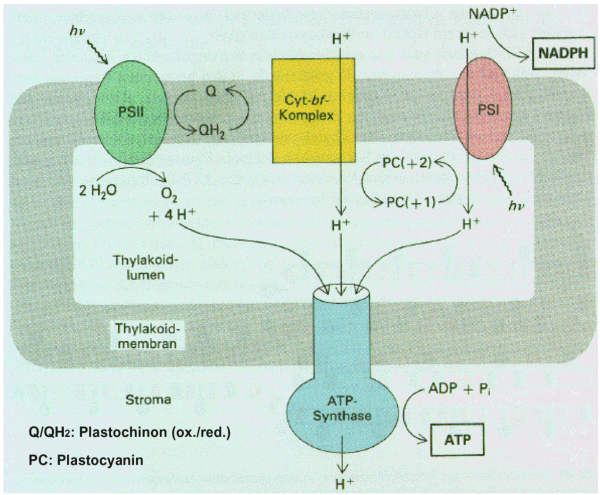

Fig.1: Scetch of the light reaction of photo-synthesis of a herbal chloroplast. The four in-volved enzyme complexes and the reactions taking place within these complexes are shown.

up additional two protons. QH_2 detaches from PS II and diffuses in the membrane. If it meets the cytochrome-bf-complex, it releases the two electrons to the complex and returns back to the PS II. Within the cytochrome-bf-complex the electrons release part of their energy and reduce a plastocyanin (PC). The complex uses the energy to pump protons from the outside to the inside of the chloroplast. The PC transports the electron to the PSI where additional light absorption excites the electrons again which are transferred to $NADP^+$. Thisway the energy-rich compound NADPH is formed (see Fig.2). As can be seen in Fig.1 the fission of water is done at the inside, the formation of NADPH at the outside of the membrane of the chloroplast. Therefore, a dissociation of charges is done by which the positive protons are accumulated at the inside. The concentration gradient is decreased by a proton channel of the ATP-synthase. Again, energy is released in this process and stored in the universal energy currency ATP. For the investigation of the function of ATP-synthesis the Nobel prize of chemistry was given in 1997 to Paul D. Boyer and John E. Walker. ATP and NADPH are used in an additional reaction cycle called "dark reaction" to form several sugars (formula $C_6H_{12}O_6$) which are used as energy reservoirs and nutrient:

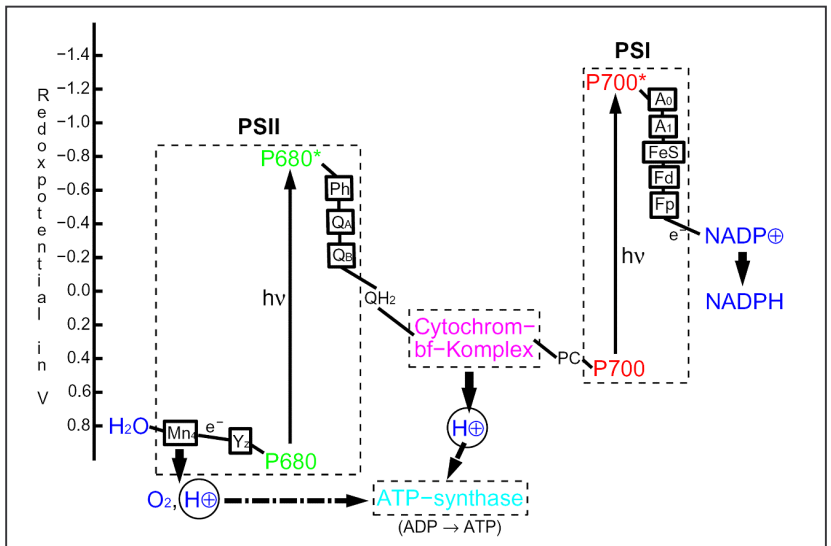

Fig.2: Potential scheme of the electron transfer of herbal photosynthesis.

$$6CO_2 + 12NADPH + 18ATP \longrightarrow C_6H_{12}O_6 + 12NADP + 18ADP + 18P$$

The "dark reaction" is also called "Calvin-cycle". Melvin Calvin got the Nobel prize in 1961 for the detailed investigation of this reaction cycle by radioactive markers.

2.2 Bacterial reaction centers

Photosynthetically active bacteria are somewhat easier to understand. Hence, the single steps are also easier to investigate. Accordingly, the bacteria of the kind *rhodopseudomonas* have only one photosynthetic reaction center (RC) being similar to the PS II of plants. It is also used to excite electrons by light. However, the electrons are not taken from water but from other molecules so that no oxygen is produced. For the determination of the three-dimensional structure of the RC of the bacterium *Rps. viridis* by X-ray crystallography, Johann Deisenhofer, Robert Huber and Hartmut Michel got the Nobel prize in 1988. In combination with numerous spectroscopic results the structure provided a significant insight in the mechanism of photosynthesis. The RC of *Rps. viridis* is the sample of the present experiment.

The RC consists of four proteins (the polypeptides L, M and H as well as a cytochrome c; see Fig.3) and several prosthetic groups (i.e. parts of the proteins which are not build of amino acids). The cytochrome c (Cyt c) has four haem-groups, each containing one iron atom.

Therefore it is also called tetra-haem cytochrome. An additional iron atom is located in the center of the complex between the polypeptides L and M. To distinguish it from the haem irons, it is called non-haem iron. The main function of the non-haem iron seems to be the stabilization of the structure because it is bound to some amino acids of L and M. It is possible, but unlikely, that this iron is involved in the electron transfer process: it can be substituted by other bivalent metal ions that do not show a transition to the 3+ state like iron. By the Cyt c the electrons are transferred to the RC. In turn, the tightly bound Cyt c is provided with electrons by a cytochrome c_2 which can diffuse through the cell and receive electrons from other compounds.

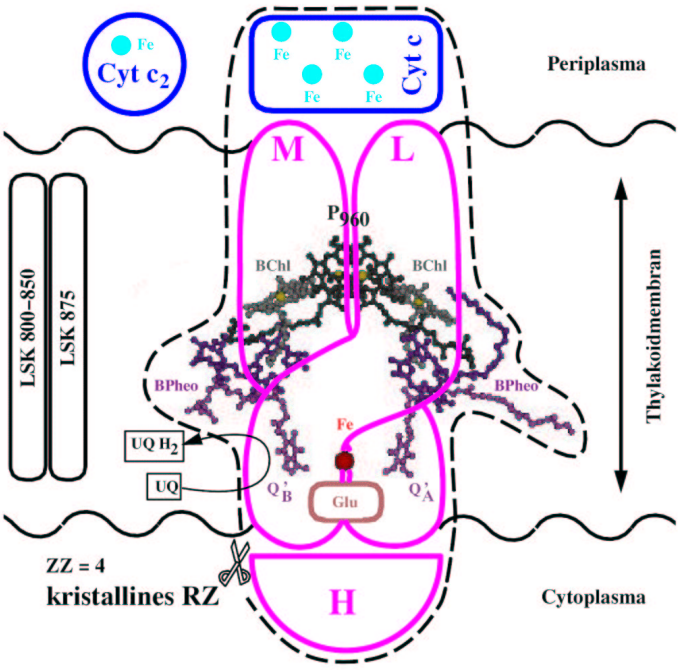

Fig.3: Schematic view of the bacterial reaction center of *Rhodopseudomonas viridis* within the membrane. The dashed area represents the part which is crystallized and forms our sample. (LSK: light harvesting complex - "amplifies" light to lower energy which afterwards can be absorbed by P960)

Within the experiment, the oxidation state and spin of the iron atoms has to be determined by Mössbauer spectroscopy. For this purpose, the natural iron which contains only 2.2% of the Mössbauer isotope ^{57}Fe was enriched in ^{57}Fe by growing up the bacteria on appropriate growing media.

3. The Mössbauer effect

In the following the Mössbauer effect is described. It was discovered in 1958 by the Ph.D. student R.L. Mössbauer which got the Nobel prize for this discovery three years later. Not so much the physical idea behind that discovery, but the numerous applications in solid state physics, metallurgy, archeology, chemistry and biology forwarded this award.

3.1 Basic concepts

For the emission and absorption of photons (γ-quanta in this case) the energy and momentum conservation is valid. Due to the momentum conservation an excited nucleus receives a recoil by the emission of a γ-quantum. The energy E_γ of that quantum has, due to the energy conservation law; to be diminished by the recoil energy of the nucleus:

$$E_\gamma = E_0 - E_R \qquad \text{with} \qquad E_R = \frac{\hbar^2 k^2}{2M}$$

$E_0 = \hbar\omega_0$ is the energy of the transition, $p = \hbar k$ is the momentum of the emitted quantum and M is the mass of the nucleus. Consequently, a quantum which should be absorbed by a

nucleus has to have an energy being E_R higher than the transition energy. That means, that a emitted quantum cannot be absorbed by the same transition due to the fact that it lacks the energy of $2E_R$ for this process. Due to the finite lifetime τ of the excited state the emission and absorption energies are not infinitely sharp. The energy distribution of emitted and absorbed quanta is a Lorentzian with a linewidth of $\Gamma = \hbar/\tau$ (given by the Heisenberg uncertainty relation). The lifetime of nuclear excited states is often so long and hence the linewidth so small, that the distribution of emission and absorption levels do not overlap and a resonant absorption is impossible.
The considerations given above are valid for free nuclei which are at rest at the time of emission and absorption. Due to their thermal energy, nuclei are not at rest. Therefore the linewidth can be thermally broadened by a gaussian distribution due to the Doppler effect. Only if this Doppler broadening is large enough to result in an overlap of emission and absorption lines, a resonance absorption can take place.
If the nucleus under consideration is part of a solid, there is a certain possibility that the momentum of recoil is transferred to the solid as a whole. Now the mass is nearly infinity in comparison to the mass of one single nucleus, the energy of recoil is practically zero and the nucleus can emit or absorb "without" recoil. This statement is valid if no lattice vibrations (phonons) are invoked. If a phonon is created, it takes the momentum of the emitted photon and a typical energy of some meV which destroys the resonance condition. The finite possibility that no phonon is created or annihilated in the resonance absorption is called the Mössbauer effect.

The amount of nuclei which emit or absorb a γ-quantum without recoil can in a classical model be calculated as follows:
Assume a nucleus emitting a electromagnetic wave of frequency $\omega_0 = E_\gamma/\hbar$ with the amplitude A_0. In addition the nucleus should vibrate with the frequency Ω (Einstein model of a solid) and the amplitude a. The emitted wave can then be written as:

$$E(t) = A_0 e^{-i(\omega_0 t + kx(t))} = A_0 e^{-i\omega_0 t} e^{-ikx(t)} \qquad \text{with} \quad x(t) = a\sin(\Omega t)$$

$k = 2\pi/\lambda$ is the wavevector of the radiation and λ is its wavelength. If one makes a Taylor-series expansion on the second exponential function and uses the equation $\sin^n(\Omega t) = (2i)^{-n}\left(e^{i\Omega t} - e^{-i\Omega t}\right)^n$ one obtains for the time dependent amplitude:

$$E(t) = A_0 e^{-\frac{k^2 a^2}{4}} e^{-i\omega_0 t} + A_0\left(-\frac{ka}{2} + \ldots\right)\left(e^{-i(\omega_0-\Omega)t} - e^{-i(\omega_0+\Omega)t}\right) + \ldots$$

It follows that the intensity of the electromagnetic wave of energy $\hbar\omega_0$ is not $|A_0|^2$ but only $|A_0|^2 e^{-\frac{k^2 a^2}{2}}$. This value corresponds to the quanta which are emitted without recoil. In return there is additional radiation shifted by the energies $\pm\hbar\Omega, \pm 2\hbar\Omega, \ldots$ (these corresponds to creation and annihilation of phonons!). For the one-dimensional harmonic oscillator, the mean square vibrational amplitude is $\langle x^2 \rangle = \frac{a^2}{2}$ and one obtains for the recoil free fraction of emitted quanta:

$$f = e^{-k^2\langle x^2\rangle} \quad ; \qquad \text{f is called Lamb-Mössbauer factor.}$$

3.2 The principle of measurement

If the emitting nuclei (source) and the absorbing nuclei (absorber) are embedded in different solids one normally cannot measure any resonance absorption. The reason is that different surroundings generate small shifting of the nuclear energy levels and therefore of the transition energies. Due to the tiny linewidth one cannot observe a resonance absorption anymore. This example illustrates, that one can in principle measure very small changes in energy levels not only due to different surroundings but also due to electric and magnetic fields (see e.g. 3.4.2).

To get a spectrometer using this enormous sensitivity, the source is fixed on a so called "drive". The drive is an electromagnetic traction unit with which the source can be moved back and forth with various velocities v. Due to the Doppler effect the emitted quanta experience an energy shift of $\Delta E = E_0 \frac{v}{c}$. Crossing a collimator the quanta reach the absorber and afterwards a detector (see Fig.4).

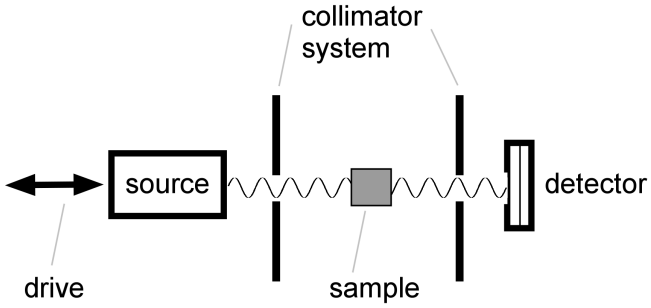

Fig.4: Schematic view of the setup for the measurement of a Mössbauer spectrum

The detector measures the number of quanta dependent on the velocity v of the source. Normally, the velocity is taken sinusoidal. If the transition energy E_a of the absorber is $E_a = E_s + \Delta E$ (where E_s is the transition energy of the source), then a minimum at the suitable velocity v is present in the Mössbauer spectrum: at this velocity less quanta reach the detector than at velocities where the reonance condition is not fullfilled. Frequently the absorber (sometime the source too) are cooled within a cryostat. Thereby the dynamical mean square vibration $\langle x^2 \rangle$ is lowered and a larger Lamb-Mössbauer factor is obtained. This way the resonance absorption is increased and the measuring time can be abbreviated significantly.

3.3 The Mössbauer isotope ^{57}Fe

The most popular isotope (from 110 Mössbauer isotopes) for Mössbauer spectroscopy is ^{57}Fe (64% of the publications). The reason for this popularity is, that its mother isotope ^{57}Co has a relatively long lifetime of 271 days before it decays by electron capture (with a yield of 99.8%). Hence, the radioactive ^{57}Co can be used for about three years with

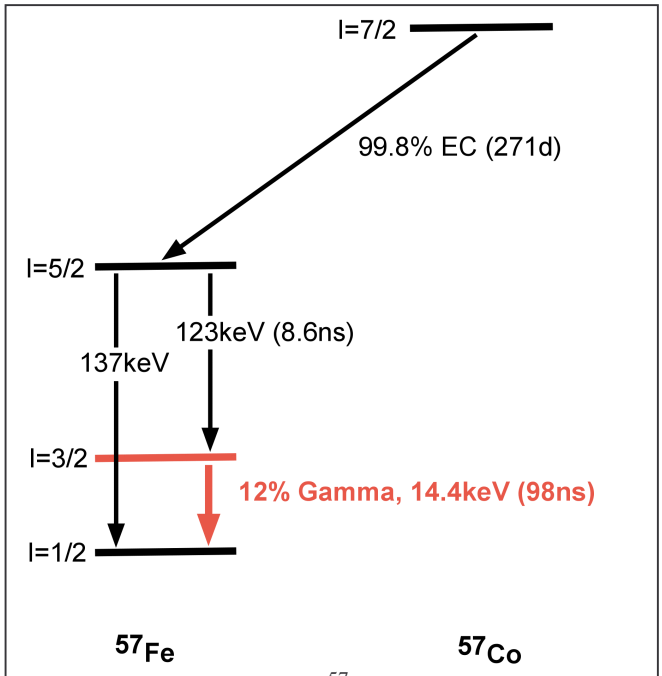

Fig.5: Energy levels for ^{57}Fe; the transition energies (in brackets the half lifetimes) are given. The Mössbauer transition is in red.

acceptable counting rates. In the source the ^{57}Co is often embedded in a rhodium matrix (^{57}CoRh source) or it is given as ^{57}CoO. A second advantage of ^{57}Fe is the relative long lifetime of the Mössbauer transition: the lifetime τ=141ns corresponds to a very small linewidth of $4.7 \cdot 10^{-9}$eV. The energy of the Mössbauer transition is 14.4keV. The complete energy diagram is seen in Fig.5.

The advantage of ^{57}Fe for biophysicists is, that iron is occurring in a large number of biomolecules. Nevertheless, due to the small sample volumes, one has to enrich the natural iron content by the Mössbauer isotope.

3.4 Theory

3.4.1 Absorption lines

The number Z of quanta which are detected at a certain velocity v of the drive is given by:

$$Z(v) = CRf_s \int_{-\infty}^{\infty} Q(v, E_s, E) e^{-\sigma_a(E_s,E) n_{Fe}} dE + CR(1 - f_s) + CU + U'$$

The constant C depends on factors like the measuring time, the geometry of the equipment and the intensity of the source. The indices s and a stand for the source and the absorber respectively. The different summands describe the following physical effects:

$CR(1 - f_s)$: Amount of quanta which had been emitted with recoil. Due to the small recoil energy and limited energy resolution of the detector they are indistinguishable in the pulse height spectrum (PHS, see 4.2 and Fig.10) from those quanta which are emitted without recoil.

CU: Radiation from the source which cannot be discriminated from the Mössbauer quanta by the detector. This part consists of neighboring X-ray lines (from the source material, lead shielding etc.) and of Compton scattering from high energy γ-rays (e.g. 136keV and 122keV for ^{57}Fe).

U´: Natural background radiation, electrical noise, ...; this amount is small against the other parts.

The radiation detected within the 14.4keV-window (see 4.2 and Fig.10) consists, therefore, of Mössbauer 14.4keV quanta R and of background quanta U. The part CR is further subdivided into CRf_s quanta which had been emitted by the source without recoil and $CR(1-f_s)$ with recoil. Whereas the subgroup "CRf_s" can be resonantly absorbed in the sample, the second subgroup can not. If the content of the 14.4keV-window is normalized one has: R+U=1.
The integral in this equation, the so called transmission integral, describes the resonant absorption. It is composed of the normalized Lorentzian emission spectrum of the source:

$$Q(v, E_s, E) = \frac{\frac{\Gamma_{nat}}{2\pi}}{\left(E - E_s\left(1 + \frac{v}{c}\right)\right)^2 + \left(\frac{\Gamma_{nat}}{2}\right)^2}$$

and the absorption by the sample (e-function) with the Lorentzian absorption cross section:

$$\sigma_a(E_a,E)=\sigma_0 f_a \frac{\left(\frac{\Gamma_{nat}}{2}\right)^2}{(E-E_a)^2+\left(\frac{\Gamma_{nat}}{2}\right)^2}$$

The constants are: Γ_{nat} , the natural linewidth of ^{57}Fe
$\sigma_0=2.56\cdot10^{-18}cm^2$, the maximum cross section of the Mössbauer level of 57Fe
n_{Fe} , the area density of ^{57}Fe within the sample
f_a , the Lamb-Mössbauer factor of the sample

The transmission, i.e. the number of detected quanta normalized to one for Doppler velocities where the resonance absorption is totally destroyed is:

$$T(v)=\frac{Z(v)}{Z(\infty)}=1-Rf_s\left(1-\int_{-\infty}^{\infty}Q(v,E_s,E)e^{-\sigma_a(E_s,E)n_{Fe}}dE\right)$$

For "thin" absorbers (these are samples with a small n_{Fe}) one can expand the exponential function into a Taylor series and truncate it after the second addend. The integral can be solved with the convolution theorem and one obtains the thin-absorber-approximation for the transmission:

$$T(v)=1-Rf_s t_a \frac{\frac{\Gamma_a}{2}\frac{\Gamma_s+\Gamma_a}{2}}{\left(E_a-E_s\left(1+\frac{v}{c}\right)\right)^2+\left(\frac{\Gamma_s+\Gamma_a}{2}\right)^2}$$

The individual linewidth for the source and for the absorber are taken as Γ_s and Γ_a respectively and may be larger than the natural linewidth Γ_{nat}. In addition all parameters which determine the thickness (i.e. the cross section) of the sample are collected into a single parameter, the effective thickness: $t_a=\sigma_0 f_a n_{Fe}\frac{\Gamma_{nat}}{\Gamma_a}$

The area of the Mössbauer spectrum in the thin-absorber-approximation can be calculated by integration over all Doppler velocities:

$$A'=\int_{-\infty}^{\infty}(1-T(v))dv=\pi Rf_s t_a\frac{\Gamma_a}{2}\frac{c}{E_s}$$

In order to eliminate the dependency from the energy of the source and unhandy numbers one changes the unit of Γ_a to mm/s by rescaling the energy:
$\Gamma_a[mm/s]=\Gamma_a[eV]\cdot c[mm/s]/E_s[eV]$. This area (in units of mm/s) is calculated by the analysis software:

$$A[mm/s]=\pi Rf_s t_a\frac{\Gamma_a[mm/s]}{2}$$

If f_s, n_{Fe} and R are known, one can extract the Lamb-Mössbauer factor f_a of the sample. Values for f_s and n_{Fe} are given below. R is a result of the analysis of the pulse height spectrum: it is the total number of events within the 14.4keV-window less the background divided by the total number of events within the 14.4keV-window (compare Fig.10).

3.4.2 Hyperfine interactions

The extremely good energy resolution of the Mössbauer spectroscopy allows to measure smallest energy shifts in the region of neV. The physical reason for such a energy shift is the electromagnetic interaction between the absorbing nucleus and the surrounding electronic structure. If one expands the interaction energy into multipoles one can distinguish different contributions. Due to the fact that there exist no magnetic monopoles, nuclei do not have an electric dipole moment and contributions of higher orders in the expansion are getting smaller rapidly, one normally classifies just the following three interactions:

a) The electrical monopole interaction (called isomer shift)
b) The electrical quadrupole interaction (results in the so called quadrupole splitting of energy levels)
c) The magnetic dipole interaction (results in the magnetic hyperfine splitting of energy levels)

a) Isomer shift

As already mentioned in 3.2, a shift of an absorption line in respect of v=0 happens, if the Mössbauer nuclei in source and absorber are embedded in different materials. This effect is called isomer shift. It depends of the electrostatic interaction of a spacious nucleus with the electrons of the atomic orbitals (see Fig.6 below). If the mean squared radius of the nucleus is named $\langle r^2 \rangle$ and if one assumes that the electron density $|\Psi(0)|$ is constant within the volume of the nucleus, the energy is enlarged by

$$\frac{2\pi}{3} Ze^2 |\Psi(0)|^2 \langle r^2 \rangle$$

compared to a point-like nucleus. Z is the charge of the nucleus, e is the electron charge. If the nucleus has a different radius in the excited state compared to the ground state (indices named e and g respectively) the transition energy is proportional to:

$$\frac{2\pi}{3} Ze^2 |\Psi(0)|^2 \left(\langle r^2 \rangle_e - \langle r^2 \rangle_g \right)$$

The electron density $|\Psi(0)|$ is different from zero only for the s-electrons. These are not directly involved in chemical reactions of the iron. However, changes in the oxidation state or in the chemical surrounding have an influence on the density distribution also of the s-electrons. If the s-electron density is different for the source, $|\Psi_s(0)|^2$, and the absorber, $|\Psi_a(0)|^2$, one obtains an isomer shift δ a follows:

$$\delta = \text{const.} \cdot \left(|\Psi_a(0)|^2 - |\Psi_s(0)|^2 \right) \left(\langle r^2 \rangle_e - \langle r^2 \rangle_g \right)$$

For iron, the isomer shift decreases with increasing oxidation number because the shielding of the 3d-electrons is decreasing.

It should be mentioned, that in addition to the isomer shift discussed above there is a second effect which shifts the absorption line, the quadratic Doppler effect. This effect is of relativistic origin and stems from the thermal motions of the atoms within the solid. It is, especially for biomolecules, very difficult to separate both parts. Therefore often the total measured value (the sum of both parts) is called isomer shift.

b) Quadrupole splitting

Nuclei of spin I ≥ 1 have an electrical quadrupole moment Q. This means, in a descriptive picture, that the nucleus is not spherical anymore. If in this case there is an electrical field gradient at the nucleus, the so far degenerated energy levels split dependent of their magnet quantum number M_I:

$$E_Q = \frac{eQV_{zz}}{4} \cdot \frac{3M_I^2 - I(I+1)}{I(2I-1)}$$

$V_{zz} = \frac{\partial^2 V}{\partial z^2}(0)$ is the electrical field gradient at the nucleus. It is obvious, that E_Q is only dependent on the absolute value of the magnetic quantum number M_I (it is degenerated in respect of the sign of M_I).

For ^{57}Fe, the groundstate has a spin of I=1/2 and has therefore no quadrupole moment. The excited state has I=3/2 and therefore $E_Q = \pm\frac{eQV_{zz}}{4}$. For simplicity one prefers sources with only one emission line; the material of the sources is therefore chosen to have no electrical field gradient. However, a quadrupole splitting ΔE_Q in a sample is an informative measure. For ^{57}Fe one obtains:

$$\Delta E_Q = \frac{eQV_{zz}}{2} \cdot \sqrt{1 - \frac{\eta^2}{3}}$$

The parameter $\eta = \frac{V_{xx} - V_{yy}}{V_{zz}}$ in called asymmetry parameter. it describes deviations of the electrical field gradient from the rotational symmetry around the z-axes. The quadrupole splitting is superposed by the isomer shift. Therefore one normally measures the center of the quadrupole duplet shifted relative to v=0 (see e.g. Fig.6).

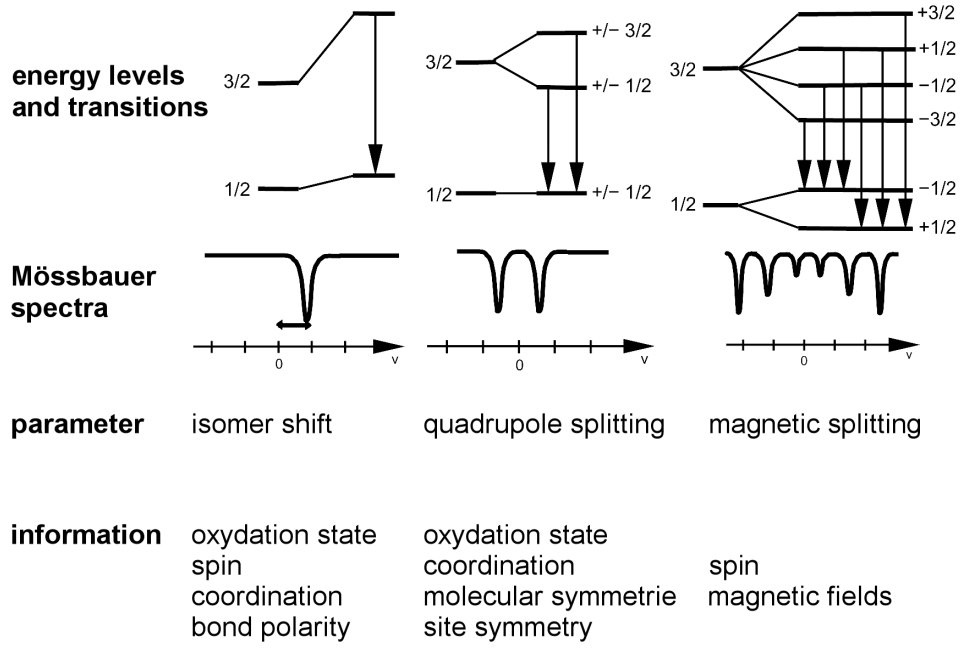

Fig.6: The hyperfine interactions

c) Magnetic hyperfine splitting

The nuclear energy levels split via an interaction of the magnetic dipole moment with a magnetic field at the nucleus (nuclear Zeemann effect). The degeneracy by M_I is totally canceled (and not only for the absolute value like in the quadrupole interaction). The magnetic field may come from the electrons of the iron atom or be applied from outside. Magnetic fields from the electrons are often only measurable at low temperatures or if a small magnetic field is applied in addition from outside to stabilize the electron spins. Otherwise spin flips would occur so fast, that the mean magnetic field tends to zero and no magnetic splitting is measurable any more. On the other side the magnetic field of ferromagnets may be so stable, that already at room temperature the magnetic hyperfine splitting is measurable. For metallic iron, e.g., one measures a 6-lines spectrum. This is obtained with respect to the selection rule for magnet transitions: $\Delta M_I = 0, \pm 1$ (see Fig.6).

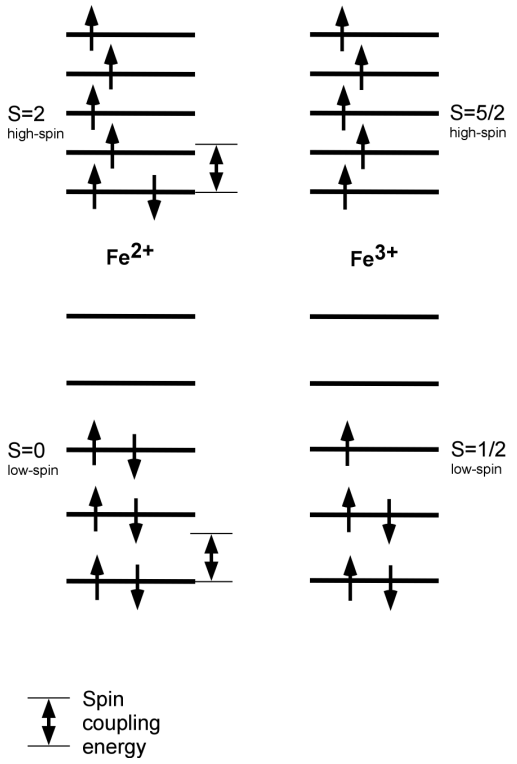

Fig.7: Scheme for iron in the high-spin and low-spin states

3.4.3. Low-spin and high-spin iron

Different oxidation states result in different distributions of the electron density in the 3d-orbitals. Different electron densities in the 3d-orbitals further on result in different electrical and magnetic fields at the nucleus so that the hyperfine parameter are different. The electronic configuration is listed in the following table:

	1s	2s	2p	3s	3p	3d	4s
Fe	2	2	6	2	6	**6**	**2**
Fe^{2+}	2	2	6	2	6	**6**	**0**
Fe^{3+}	2	2	6	2	6	**5**	**0**

Tab.1: Electronic configuration of iron in several oxidation states

The 3d shell consists of 5 orbitals which are in iron compounds normally not degenerated. Hence, one has several possibilities for the total spin. If the energetic splitting of the orbitals is small compared to the spin coupling energy, the orbitals are filled as long as possible (following Hund's rule with) each with one electron. For Fe^{2+} this rule results in a total spin of S=2 and for Fe^{3+} of S=5/2 (compare Fig.7). The iron in this cases is often called high-spin iron. If the splitting of the orbitals is larger than the spin coupling energy, firstly the lowest orbital will be filled with two electrons before the next higher orbital is filled (and so forth). For Fe^{2+} one gets now S=0 and for Fe^{3+} the total spin is S=1/2. This states are called low-spin iron. The four possibilities are shown in Fig.7.

In the exercise one should determine the different iron states in bacterial RC by their hyperfine parameters. The following table gives some typical values for the isomer shift and the quadrupole splitting of the different configurations of the iron in proteins.

Tab.2: The table gives the values of the isomer shift and the quadrupole splitting in mm/s. The values are typical for iron in biological samples at liquid nitrogen temperature. The isomer shift is given in respect to a ^{57}CoRh-source.

	isomere shift	quadrupole splitting
Fe^{2+} ls	0.3	1.0
Fe^{2+} hs	1.0	2.0
Fe^{3+} ls	0.1	2.0
Fe^{3+} hs	0.3	0.7

4. The realization of the equipment and the execution of the exercise

4.1 The equipment

The equipment is schematically shown in Fig.8. A 57CoRh-source is mounted on a velocity transducer which is driven by a sinusoidal voltage at an actuation coil in a magnetic field. In a pick-up coil a induction voltage is induced which is provided for the control electronics to obtain the actual velocity. If the course of the velocity is deviating from the desired sinus (e.g. by external vibrations) the driving voltage is changed accordingly. The deviations from the wanted velocity will always be less than 5‰. A lead shielding collimates the radiation to a beam which hits the sample in an cryostat. The cryostat is filled with liquid nitrogen to ensure a temperature of the sample of 80K. The quanta which are not absorbed by the sample are detected in a xenon-gas filled proportional counter. The detector pulses are gained and converted to rectangular pulses (logical "1" for a computer). The pulses are added into the memory of a multi channel analyzer dependent of the actual velocity of the drive and source. The Mössbauer spectra obtain in such a way can be transferred to a Computer for further processing. Fig.9 gives a block diagram of the control and amplifier electronics.

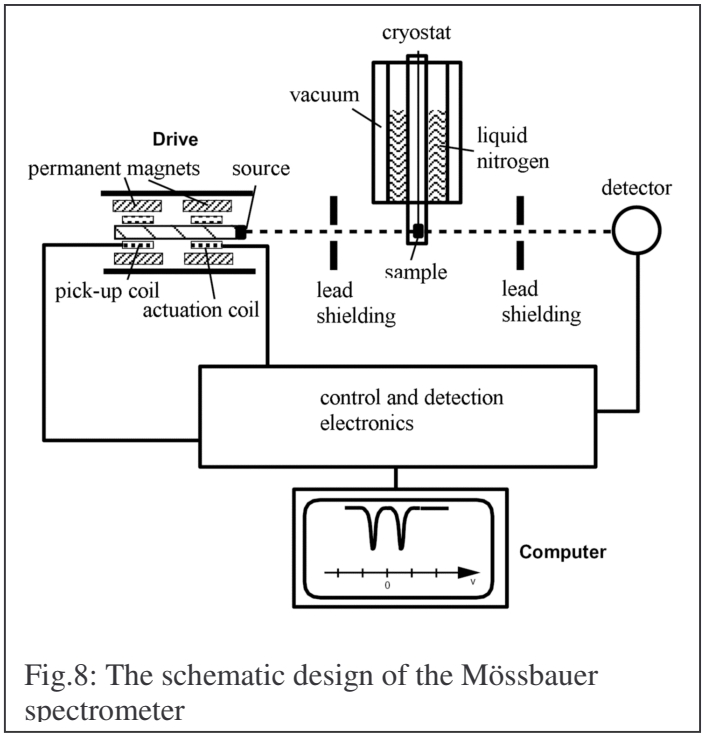

Fig.8: The schematic design of the Mössbauer spectrometer

4.2. Execution of the experiment

a) velocity calibration

Before starting with the actual measurement at the protein, one has to determine the velocity (i.e. the maximum of the sinusoidal lapse) with an calibration. This is done with a known

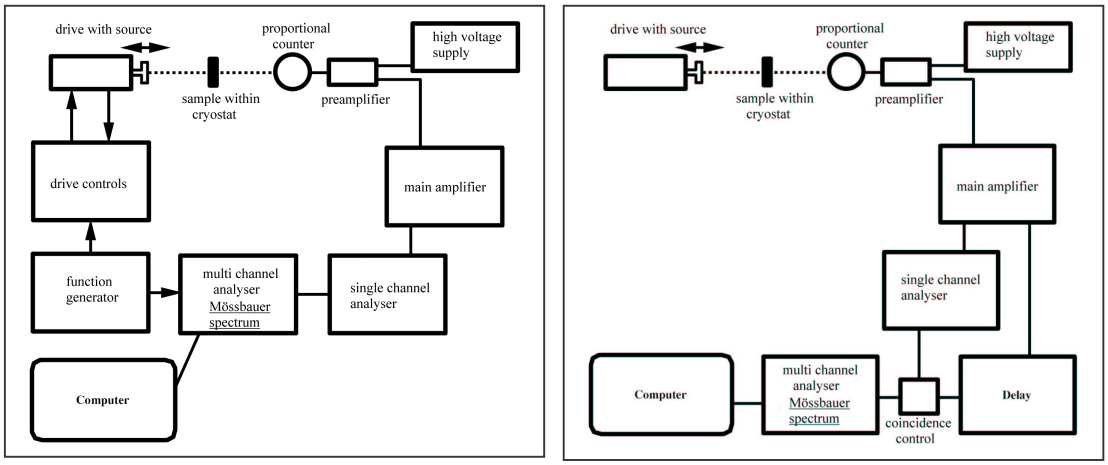

Fig.9: Block diagramm of the electronics for the measurement of a Mössbauer spectrum (left) and of the puls hight spectrum (right).

absorber, sodium nitro prussid, SNP, which has a very well known quadrupole splitting of 1.7032mm/s. This absorber can be attached to the outside of the cryostat. The analysis software demands the maximum velocity as an input parameter. This can be varied as long as the fitted output for the quadrupole splitting is correct (this can simply be done by the rule of proportion). The optimal maximum velocity for the measurement at the RC-crystals is between 5 and 6mm/s. An input voltage of 300mV0s at the driving unit corresponds to a velocity of about 9.4mm/s. The input voltage can be picked up at the driving unit and displayed on an oscilloscope.

b) the protein sample

For the measurement of the protein, the sample with the RC-crystals can be brought into the cryostat by a sample rod. The measurement starts after the sample reached the desired temperature of liquid nitrogen. Due to the low iron content the measurement will take some hours or days till a sufficient statistics has been collected. Therefore the absorption spectrum of the preceding group will be used for analysis and the just started measurement will by used by the next group.

c) the pulse height spectrum, PHS

Before one can start the actual measurement, one has to take care that the counting electronics is triggered on the Mössbauer quanta and not on some background garbage. For this purpose the pulse height spectrum has to be collected. The PHS is the spectrum of the incoming radiation in dependence of their energy. For this purpose one needs a proportional counter a detector. In Fig.10 an example of a PHS is shown. In addition to the 14.4keV Mössbauer line (around channel 350 in Fig.10) one finds additional lines at other energies. These lines are mainly the 6.4keV K_α X-ray line of iron and two X-ray lines of rhodium at 20.2keV (K_α) and 21.6keV (K_β). For a optimal background-free Mössbauer spectrum, only the 14.4keV radiation should be counted in the multi channel analyzer (MCA). To ensure this, a single channel analyzer checks the incoming puls height. Only if a puls

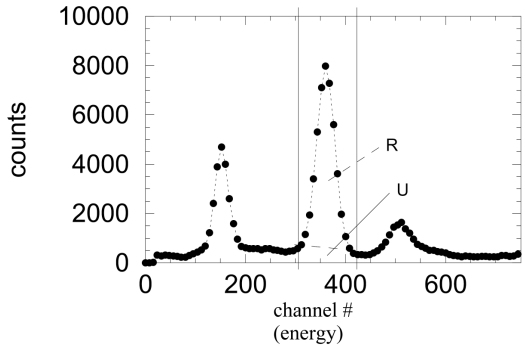

Fig.10: Pulse hight spectrum of a ^{57}CoRh source

coming from the detector is lying in an energy window between a lower and a higher level (which can be tuned appropriate) the MCA is triggert via a coincidence control. For the analysis of the Mössbauer absorption spectrum one needs the part R of Mössbauer quanta within this energy window. This is calculated by a analysis software.

5. Questions and duties

5.1 Questions

The following questions should be reviewed before the experiment starts:

1. Explain the importance of photosynthesis.
2. How is a proportional counter constructed and how is it working (in contrast e.g. to a Geiger-Müller counter)?
3. Explain the conservation of energy and momentum in connection with the recoil free emission and absorption of photons.
4. How can one measure the dynamics of molecules in solid state by the Mössbauer effect? What is the dependence of the temperature?
5. What is the natural linewidth? What is a homogeneous, what is a inhomogeneous linewidth? What is the reason for a line broadening?

5.2 Duties

The following points should be worked on during the experiment and scripted in the elaboration:

1. Determine the maximum velocity of the drive by analyzing the SNP spectrum.
2. Determine the hyperfine parameters and the fraction of the several iron species of RC in the Mössbauer spectrum. With the help of table 2: Determine the oxidation state of the several iron species.
3. What is the fraction of the non-haem iron (in the high-spin state) in comparison to the cytochrome iron? What do you expect theoretically?
4. Calculate the mean square displacement $\langle x^2 \rangle$ of the several iron species by their area in the Mössbauer spectrum. What can one tell about the strength of the binding of iron within the oxidized and reduced state in cytochrome?

Some parameters you need for the calculations:

$$f_s = 0.65,\ n_{Fe}^{ox} = 2.61 \cdot 10^{17}\,cm^{-2},\ n_{Fe}^{red} = 8.04 \cdot 10^{16}\,cm^{-2},\ n_{Fe}^{non-haem} = 9.04 \cdot 10^{16}\,cm^{-2}$$

6. Literature

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