M. Gregušková, *J. Cirák, J. Novotný and I. Černohorský,
Slovak Academy of Sciences, Institute of Experimental Pharmacology and *Department of Nuclear Physics and Technics Slovak Technical University, Bratislava, Czechoslovakia.

INVESTIGATION OF IRON-CONTAINING COMPLEXES OF DEOXYRIBONUCLEIC ACID NUCLEOSIDES BY MÖSSBAUER SPECTROSCOPY

The structure of the deoxyribonucleic acid (DNA) molecule is generally accepted to consist of a long polynucleotide helix. Each helical coil is made up of alternate sugar 2-deoxy-D-ribose and phosphate groups. A side group containing either a purine or a pyrimidine base is attached to each sugar. Two purines, adenine and guanine, and two pyrimidines, cytosine and thymine, are commonly present. The two coils are joined together by hydrogen bonds between a purine base on one coil and a pyrimidine base on the other. The specific association of the purine and pyrimidine bases in the nucleic acids is a property which provides the basis for the storage, transmission and expression of genetic information. This fundamental role is explained by the specific complementarity of the hydrogen-bonding geometry of adenine with thymine, and guanine with cytosine. The base ratio A+T/G+C varies with the source of DNA. For DNA isolated from calf thymus it is about 1.25. The bases adenine, guanine, thymine and cytosine have a planar structure and are arranged in pairs in the centre of the molecule of DNA between the two helices. All these constitutents of DNA are separately available for study /10/.

It is well established by the method of activation analysis that DNA contains some amount of metallic atoms e.g. Cu, Mg, Fe, and others. Until now neither their function, nor their localization were fully elucidated in polynucleotide rings of DNA /1/. On the basis of published data and our suggestions, we attempt to study the role of iron ions in DNA by the method of Mössbauer spectroscopy. This method apart from some limitations (measuring of Mössbauer spectra of biological materials in low-viscosity solutions at room temperature), is valid because it allows to investigate not only the role of iron ions in DNA from the position of its oxidation state as well as changes in the oxidation state of iron ions due to some physical exogeneous factors, but also the localization of iron ions. Studies of interactions of nucleic acids with metallic ions are not only of considerable biological interest, but they also have added to the knowledge of the structure of DNA. From the pharmacological point of view these studies are important for the investigation of the inhibitory role of some antibiotics in DNA-dependent processes "in vitro" and within the cell, especially of the role of divalent cations (Mg^{2+}, Fe^{2+}) for these interactions /4/.

In order to obtain the sufficient density of iron atoms in the sample for Mössbauer spectroscopy, to maximize the resonant absorption and to minimize the fractional uncertainity in it, we have performed our experiments on complexes of DNA and complexes of nucleosides with ferric and ferrous ions. These investigations depend upon the concentration of iron ions, upon the ionic strength, the temperature, also upon the nature and space arrangement of the neigbouring atoms of iron ion in the complexes.

Our experiments with Mössbauer spectroscopy on nucleic acids and nucleosides were performed by transmission geometry at room temperature (300 K) on lyophilized samples and at liquid nitrogen temperature (77 K) on frozen solutions of complexes. Similar experiments were performed on UV-irradiated samples before measuring the Mössbauer spectra and with concomitant UV-irradiation of the samples during the measuring the spectra. The UV-radiation source was a high pressure mercury vapour lamp with high emission power in the region 280 - 315 nm. The samples were introduced in perspex tubes with a quartz window. The thickness of quartz wall was 0.8 mm because of preventing the loss of transmitting gamma beam.

Abbrevations used in this paper: DNA, deoxyribonucleic acid; A, adenine; T, thymine; G, guanine; C, cytosine; UV, ultraviolet; IS, isomer shift; QS, quadrupole splitting.

In the table I we demonstrate the basic data: the iron content per cm^2 in the samples, the ratio of iron atoms and nucleosides molecules, pH of the solutions and the parameters of Mössbauer spectroscopy. The values of the isomer shift are given with respect to Pd(Co) source. Figure 1 shows the typical examples of Mössbauer spectra of the ferric ions complexes of nucleosides at room temperature. The quadrupole splitting is found in all spectra with the exception of thymidine. It means that the formation of the complexes has not resulted in a change of the oxidation state of the iron ions. Furthermore, one can see the decreasing of the isomer shift and increasing of the quadrupole splitting in all complexes, with respect to the source of iron ions (iron III chloride). UV-irradiation of the samples prior to the measurements of Mössbauer spectra has not resulted in any changes in the spectra of the sample under investigation. In figure 2 we demonstrate the spectra of ferrous ion complexes of nucleosides. In these spectra we can estimate the decrease in the isomer shift and the increase in the quadrupole splitting with respect to iron ions source(iron chloridtetrahydrate), too. The UV-irradiation of the samples we have demonstrated in the previous figure in the course of 7 hours prior to the measuring of Mössbauer spectra can be seen on the figure 3. The obtained spectra can be separated into two independent quadrupoles, one of them corresponds to ferrous and the other one to ferric ion, respectively.

On the basis of our present results we can assume that UV-irradiation of the samples had resulted in changes of the oxidation state of iron ions. These changes have not obtained in the adenosine spectrum, which is in agreement with published data concerning the stability of the nucleosides against UV-irradiation /6/. In figure 4 we demonstrate DNA spectrum with ferrous ions. (DNA isolated from calf thymus) As can be seen from the spectrum, iron bonding has resulted in the change of its valency, iron ion is embeded in two oxidation states. The ratio of ferrous/ferric ions is about 2/3. Further we would like to demonstrate the time dependence of this ratio. UV-irradiation of the samples in course of 17 hours prior to measurements of Mössbauer spectra has resulted in the ratio ferrous/ferric ions 1/1, but on the other hand the measurements of the same sample 7 days after single exposition (the sample was kept in evacuated exicator chamber) has shown the ratio of 1/2.

At present there exist two opinions concerning which atoms of macromolecule of DNA play the role of iron ligands. According to the first, iron ion is bonded on purine bases mainly on six-aminogroup in adenosine and on the nitrogen in the seventh position, and in guanoside on 6-ketogroup and nitrogen in position 7. According to the second hypothesis oxygen from the phosphoric acid is assumed to be another known ligand (figure 5).

We suggest that the DNA structure may be extended, and the metallic ions could intercalate between successive base pairs. The interatomic distances between the atoms in a single hydrogen bond range from about 2.8 to 3.0 Å. The diamters of Fe^{3+} and Fe^{2+} ions are about 0.67 and 0.83 Å, respectively. Denaturation of DNA is achieved by the interruption of hydrogen bonds in the pairs of complementary bases. The proper cause of denaturation of DNA in acid surrounding is the amine-imine tautomerism of adenine, the imine form is no longer able to couple with thymine but may form a hydrogen-bonded pair with cytosine. The basis of the hypothesis concerning the role of iron ion in DNA is that is has the same denaturation properties on DNA as an acid surrounding. We assume that if the ferric ions are bonded on the aminogroup of adenosine it results in the loss of its properties as the donor in hydrogen bond. Previous findings have confirmed that ferrous iron ions stabilize DNA, and the ferric ions accelerate its denaturation (measuring melting curves of DNA) has been stated on the facts mentioned above in order to explain its function in the DNA molecule.

Acknowledgment

We would like to thank Ing T. Tóth for many helpful discussions during the course of this work.

References

/1/ V.I. Goldanskij, Chimičeskoje primenenia Mössbuaerovskoj spektroskopii, Izd."Mir", Moskva, 432-435 (1970)

/2/ N.N. Greenwood and T.C. Gibb, Mössbauer Spectroscopy, Chapman & Hall Ltd., London, pp. 112-187 (1971)

/3/ V.I. Ivanov, Biofizika, 10, 11-15 (1965)
/4/ B. Pullman, Molecular Associations in Biology, Academic Press, pp.21-298 (1968) New York
/5/ B. Pullman, Quantum Aspects of Heterocyclic Compunds in Chemistry and Biochemistry. The Israel Academy of Sciences and Humanities, Jerusalem, pp. 292-307 (1970)
/6/ B. Pullman, and A. Pullman, Quantum Biochemistry, Academic Press, pp.23-162(1963)New York
/7/ G.D. Small, and R.B. Sparks, Analytical Biochemistry, 41, 115-125 (1971)
/8/ D.C. Swinton, Photochemistry and Photobiology, 17, 361-375 (1973)
/9/ D. Ward, E. Reich, and I.H. Goldberg, Science, 149, 1259 (1965)
/10/J.S. Wyard, Solid State Biophysics, McGraw Hill Book Comp., New York, pp. 74-82 (1969).

TABLE I

Basic data for the samples

No	Sample	Temp.	mg Fe^{57} / cm^2	pH	No mol. bases on 1 at. Fe^{57}	Irrad. h	IS - A (mm/s)	QS - A (mm/s)	Γ - A (mm/s)	IS - B (mm/s)	QS - B (mm/s)	Γ - B (mm/s)
1	$Fe^{57}Cl_2$	R	4.250				1.182	3.085	0.767			
2	$FeCl_2 \cdot 4 H_2O$	R	0.200				1.070	2.930	0.260			
3	$FeCl_3$	R	0.190				0.230	0	0.300			
4	Adenosine + $FeCl_3$	R	0.190	2.04	422		0.190	0.670	0.520			
5	Guanosine + $FeCl_3$	R	0.190	1.95	393		0.210	0.660	0.530			
6	Cytidin + $FeCl_3$	R	0.179	2.27	468		0.170	0.730	0.530			
7	Thymidine + $FeCl_3$	R	0.179	1.95	468		0.140	0	1.020			
8	Adenosine + $FeCl_3$	R	0.190	2.04	422	7	0.160	0.650	0.500			
9	Guanosine + $FeCl_3$	R	0.190	1.95	393	11	0.190	0.640	0.560			
10	Cytidin + $FeCl_3$	R	0.179	2.27	468	7	0.170	0.670	0.550			
11	Guanosine + $FeCl_2$	R	2.250	3.80	102		0.088	0.898	0.507	1.062	2.325	0.782
12	Guanosine + $FeCl_2$	R	2.250	3.80	102		0.072	1.239	0.625	1.223	2.724	0.850
13	Guanosine + $FeCl_2$	R	2.250	3.80	102	7	0.146	0.920	0.663	1.035	2.348	0.789
14	Adenosine + $FeCl_2$	R	4.250	3.60	41		1.005	2.374	0.893			
15	Adenosine + $FeCl_2$	R	4.250	3.60	41		1.131	2.896	0.918			
16	Adenosine + $FeCl_2$	R	4.250	3.60	41	7	1.210	2.420	0.930			
17	DNA + $FeCl_2$	R	0.184	4.60	135		0.210	0.560	0.440	1.070	2.980	0.300
18	DNA + $FeCl_2$	R	0.118	4.60	135	17	0.190	0.580	0.470	0.950	2.960	0.330
19	DNA + $FeCl_2$	R	0.184	4.60	135	16*	0.190	0.610	0.520			
20	DNA + $FeCl_2$	R	0.118	4.60	135		0.230	0.560	0.440	1.090	2.960	0.280

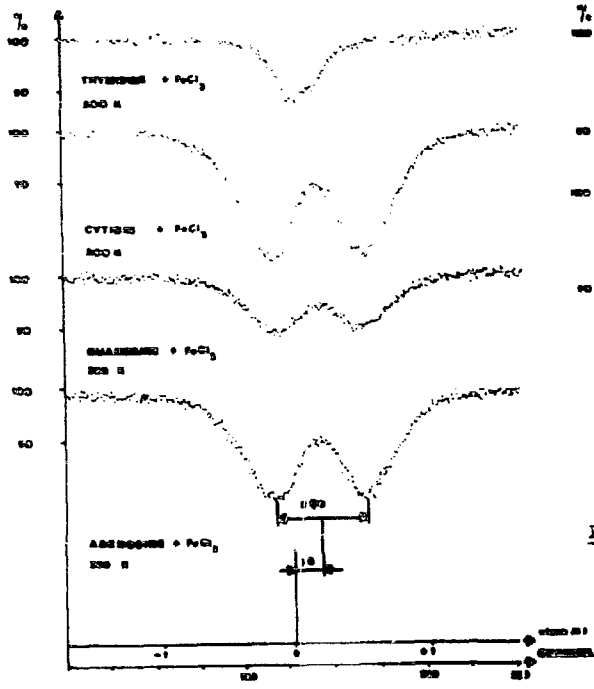


Fig.1. Mössbauer spectra of some Fe^{3+} complexes

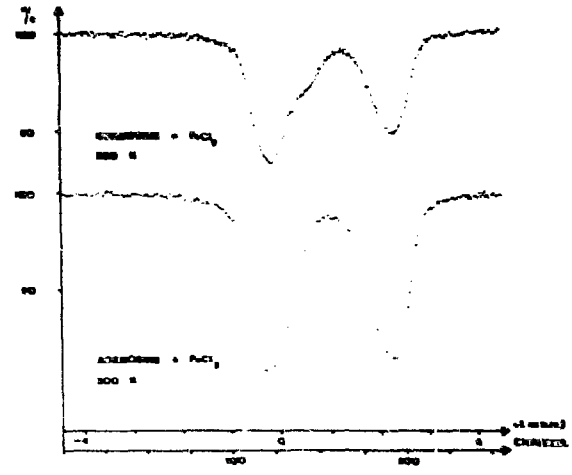


Fig.2. Mössbauer spectra of some Fe^{2+} complexes

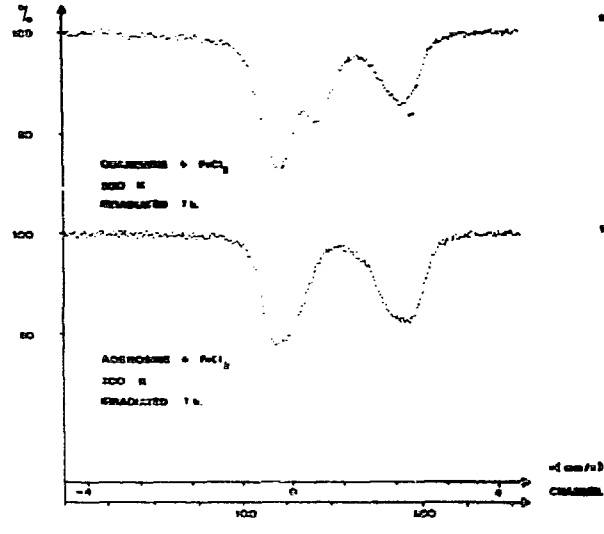


Fig.3. Mössbauer spectra of irradiated Fe^{2+} complexes

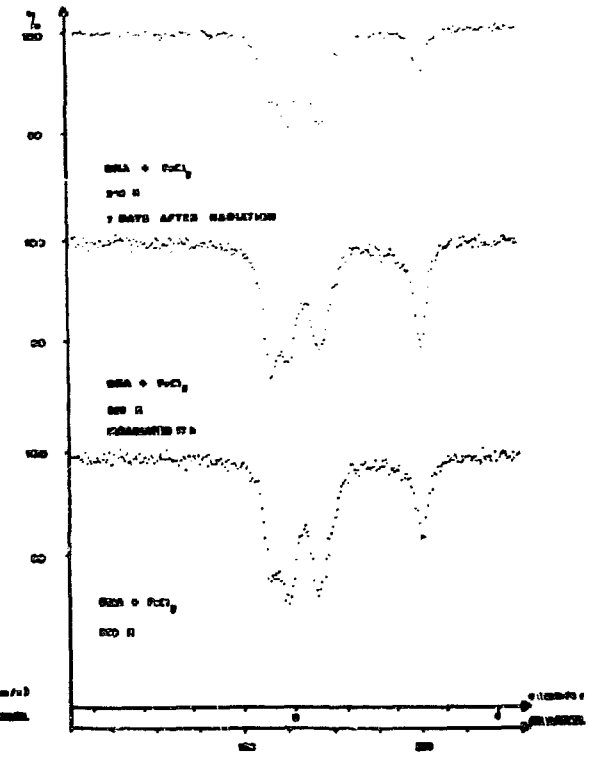
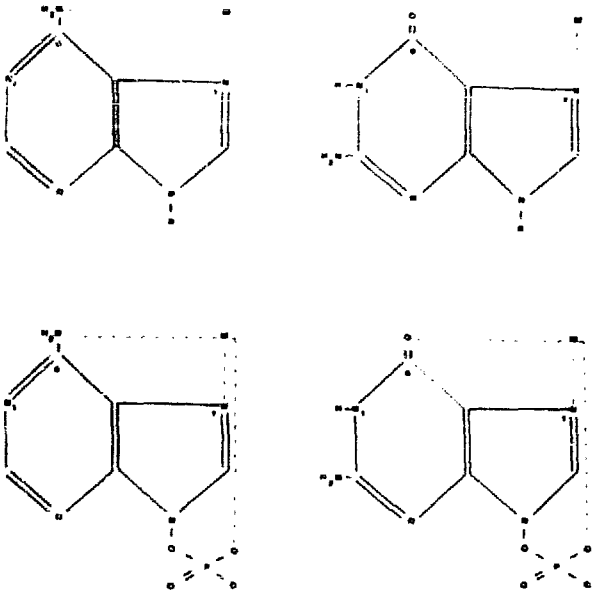


Fig.4. Mössbauer spectra of irradiated DHA + $FeCl_2$ complexes

Fig.5. Hypotheses of iron ligands in complexes with DNA



P. Parak, W. Zgorzalla, H. Eichler, A. Mayer. G.M. Kalvius,
Physik Department der Technischen Universität München, Garching, FRG.
K. Gersonde. M. Breitenbach, H.E. Schleak,
Institut für Physiologische Chemie und Physikochemie d. Christ. Albrechts Universität, Kiel, FRG.

MÖSSBAUER AND EPR SPECTROSCOPY OF ^{57}Fe IN BACTERIAL FERREDOXIN

Ferredoxin of the bacterial clostridium pasteurianum was prepared. This enzyme has a molecular weight of 6000 /1/ and containe two clusters consisting of 4 iron and 4 sulfur atoms each. Figure 1 shows the structure of the clusters as determined by x-ray analysis /2/. These clusters were removed by chemical means /3/ and replaced by clusters containing 80 % enriched ^{57}Fe.

For EPR measurements ative and ^{56}Fe reconstituted ferredoxin samples were used giving both the same results.

EPR spectroscopy on Fd_{oxi} at 4.2 K yields S = 0. Figure 2 shows the Mössbauer spectra of Fd_{oxi} at 4.2 K without applied magnetic field. The spectrum can be fitted by two quadrupole doublets of $1/2e^2q_1Q$ = 1.34 mm/s and $1/2e^2q_2Q$ = 0.72 mm/s and an intensity ratio of roughly 2 : 1. Since there are 8 Fe atoms in the molecule with slightly different local symmetry one should actually fit eight quadrupole doublets to the spectra. Within the limited resolution of the spectra this will not provide additional information.

The quadrupole splitting shows little temperature dependence. This fact together with the magnitude of the splittings and the isomer shift leads to the conclusion, that we have Fe^{3+} in a high spin state in the cluster. To obtain a resulting spin S = 0, as reqired from EPR measurements, one has to assume antiparallel coupling of the iron spins. This is schematically shown in figure 1. Actually we treat each of the two clusters as an entirety with a molecular wave function with S = 0. On application of 20 kG external field we find in Fd_{oxi} only the direct interaction with H_{ext} in addition to the quadrupole splitting leading to a small line broadening. This result is in keeping with the assumption of S = 0 state.

EPR spectroscopy on Fd_{red} at 4.2 K yields S = 1/2. Figure 3 shows the Mössbauer spectrum of Fd_{red} at 4.2 K without external magnetic field. The spectrum can be fitted by a quadrupole doublet with $1/2e^2qQ$ = 1.54 mm/s. The experimental line width of Γ_{exp} = 0.91 mm/s reflects