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THE INFLUENCE OF UV-RADIATION ON Fe-DNA COMPLEXES AS MEASURED BY MÖSSBAUER SPECTROSCOPY

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Résumé. — Les spectres Mössbauer des complexes organométalliques (6-aminopurine-Fe, adénosine-3'-phosphate-Fe) et (guanosine-3'-phosphate-Fe) ont été étudiés sur des échantillons bruts et irradiés aux UV. Le déplacement du maximum d'absorption dans les spectres optiques et les changements observés dans les paramètres des spectres Mössbauer des complexes précités ont permis de supposer que l'interaction dans les échantillons irradiés aux UV a lieu dans la partie de la base organique complexe au contact direct des ions fer.

Abstract. — The Mössbauer spectra of organo-metallic complexes (6-aminopurine-Fe, adenosine-3'-phosphate-Fe and guanosine-3'-phosphate-Fe) of native and UV-irradiated samples have been studied. The shift of absorption maximum in light spectrum and the identified changes of Mössbauer spectra parameters of the above mentioned complexes show that the interaction of UV-irradiated samples takes place at that part of the complex organic base is in direct moiety with iron ions.

In the present study we have been interested in the following water solution complexes :

1. 6-aminopurine-Fe.
2. adenosine-3'-phosphate-Fe.
3. guanosine-3'-phosphate-Fe.

The organic parts of the above mentioned three complexes consists of purine skelet on except 6-aminopurine has an additional amino group, adenosine-3'-phosphate amino group and sugar component and guanosine-3'-phosphate has an additional keto group and sugar part [1].

The ability of metallic ions of transitional group to create complexes with the bases and nucleosides of deoxyribonucleic acid, where complexes studied in the present work can be also included, at the present have been subjected to intensive study concerning the role of the trace amounts of metallic ions with these substances in secondary and tertiary structure of deoxyribonucleic acid [2, 3].

The complexes studied have been prepared under sterile conditions calculated as gramequivalent water solutions at pH range of 2-4. $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ was used as a source of inbonded iron ions. The values of the absorption of UV-radiation for the obtained solutions of the complexes have been measured in the range of wave length of 280-315 nm.

When comparing the absorption spectra of the complexes with those obtained for water solutions of pure organic substances (6-aminopurine, adenosine-3'-phosphate and guanosine-3'-phosphate) and absorption spectrum of $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ dissolved in water, we

have seen the shift of the character of the area of absorption maximum for pure organic substances compared to that obtained for $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ dissolved in the water, viz. 300 nm.

The Mössbauer spectra of all the complexes under investigation have been measured at a temperature of 80 K immediately after their preparation and/or also after UV-irradiation at the range of 280-315 nm ; (the UV-irradiation time of all the complexes was the same, 7 hrs.).

Up till now many models have been proposed explaining the possibility of metallic ions binding to the basic groups of nucleosides of deoxyribonucleic acid, however a characteristic feature of purine bases is the presence of two ligands. They are 6-amino group and nitrogen in position 7 for both 6-aminopurine and adenosine-3'-phosphate, and 6-keto group and nitrogen in 7th position in guanosine-3'-phosphate. For nucleosides present in deoxyribonucleic acid we can also assume the presence of the other ligands in the phosphoric acid moiety.

At the present time all the known models explaining the structure of complexes of nucleosides and bases with metallic ions differ from each other according to the coordinating number and space configuration of the components [4, 5].

Taking into account the ability of transitional ion for maximum coordinating for the obtained complexes, the most probable are such structures, when into coordination with metallic ion, in our case iron ion, the more molecules of organic substances can offer always two ligands.

For the above mentioned reasons and also as stated by Jezowska-Trzebiatowska and Kozłowski (1973) we assume that the most probable arrangement of the 6-aminopurine with metallic ion complex is depicted in figure 1. On the other hand guanosine-3'-phosphate-Fe complex has got a similar arrangement except that the keto groups and nitrogen atoms are responsible for ligands.

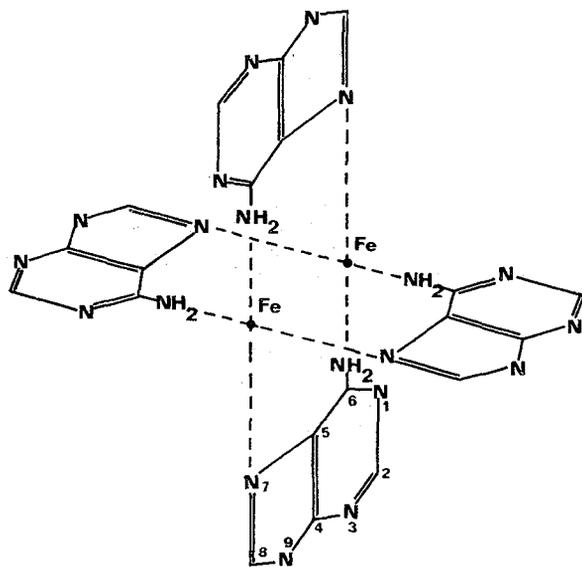


FIG. 1. — The structure of 6-aminopurine-Fe complex.

IN THESE STRUCTURES TWO POSSIBILITIES CAN BE ASSUMED :

1. In the first coordination sphere of iron ion the ligands present from four molecular residues of organic substance may be arranged in such a way that iron ion is situated on the line of two different ligands, that means amino group and nitrogen ion $N-Fe-NH_2$.

2. In the first coordination sphere the ligands are arranged in such a way that the central iron ion is bound with two ligands of the same type, namely either $N-Fe-N$ or $NH_2-Fe-NH_2$.

Taking into consideration the first approximation we can visualize the four ligands of iron ions as electric point charges, from which the difference in the values of the intensity of electric field can be calculated which is created by them in the place of iron ion ; and also similar difference in the value of the gradient of this field in near surrounding of iron ion between arrangements according to the first and second possibility.

Qualitative and quantitative description of the complexes is rather simple for the substances having known and homogeneous first coordination sphere of ligands and are present in the solid state. On the other hand, for the solutions of the complexes we can practically always presume the presence of nonhomogeneous coordination sphere created as a result of

substitution processes, e. g. interaction of substance with molecule of the solvent.

When assuming the proposed structure demonstrated on figure 1, in all the complexes studied in the present work, we have to suppose the coordination sphere of central iron ion to be nonhomogeneous from the point of the qualitative differences of some ligands. The obtained parameters of Mössbauer spectra are demonstrated on table I. The isomer shifts values of lines positions are expressed with respect to $Co^{57}Pd$ as the source.

THE COMPARISON OF NATIVE SAMPLES : WATER SOLUTION COMPLEXES-LYOPHILIZED COMPLEXES.

1. The changes of spectrum character for 6-aminopurine-Fe and guanosine-3'-phosphate-Fe have been obtained. No changes have been seen for adenosine-3'-phosphate-Fe.

2. We cannot compare the changes in the intensity of the lines due to different volumes/thickness of the samples of the solution samples and lyophilized samples. (Volumes of solutions \gg volumes of lyophilized samples.)

The different parameters of « the density » of the samples as expressed in $mg Fe^{57}/cm^2$ are present, however the ratio between the amount of molecules of organic substance and iron ions of the samples were stable.

3. In all the cases the changes of isomer shift and quadrupole splitting are due most probably to the difference obtained in the presence of solvent molecules and this might lead to the difference of magnitude of asymmetry of internal electric field.

4. The decline in Γ_{exp} parameters for the lyophilized samples in all the cases is expressed.

When we even presume that in both cases, water solution samples and lyophilized samples, the identified lines might resulted due to superposition of more nonidentified lines, the high Γ_{exp} values for the spectra of water solution samples can be explained as non-resonant processes of γ absorption due to their larger volume/thickness as compared with similar lyophilized samples.

COMPARISON OF UV-IRRADIATED SAMPLES : WATER SOLUTION COMPLEXES-LYOPHILIZED COMPLEXES.

Changes in the spectrum character of all three complexes when comparing water solution and lyophilized samples spectra have been found. In the case of water solution complex, guanosine-3'-phosphate-Fe, magnetic structure of the spectrum appeared, the magnitude of internal magnetic field of 490 kG was estimated. As previously stated we have seen similar changes of all the spectrum parameters, the position of resonance lines, their intensity and width.

In conclusion, when comparing the obtained parameters of the same complexes before and/or after exposure to UV-irradiation we can say that the cha-

TABLE I

Parameters of Mössbauer spectra of native organo-metallic complexes

Sample	Position of lines (mm.s ⁻¹)	Intensity (%)	δ (mm.s ⁻¹)	Δ (mm.s ⁻¹)	Γ_{exp} (mm.s ⁻¹)
A ₁	0.195 ± 0.061 4 0.494 ± 0.032 4	0.290 ± 0.020 4 0.570 3 ± 0.020 4	0.195 ± 0.061 4 0.494 ± 0.032 4	—	1.25 ± 0.043
A ₂	0.234 ± 0.063 2	8.832 ± 0.043	0.234 ± 0.632	—	0.692 ± 0.043
B ₁	0.074 3 ± 0.037 8 0.626 ± 0.020 8	0.476 ± 0.018 0.571 ± 0.018	0.350 ± 0.216	0.275 ± 0.021 6	0.776 ± 0.029
B ₂	- 0.233 ± 0.031 0.603 ± 0.055	9.325 ± 0.094	0.190 ± 0.032	0.413 ± 0.032	0.520 ± 0.038
C ₁	0.354 ± 0.038 3	0.638 ± 0.043	0.354 ± 0.038	—	1.15 ± 0.029
C ₂	- 0.192 ± 0.034	8.325 ± 0.083 4	- 0.192 ± 0.034	—	0.55 ± 0.020

Parameters of Mössbauer spectra of UV-irradiated organo-metallic complexes

Sample	Position of lines (mm.s ⁻¹)	Intensity (%)	δ (mm.s ⁻¹)	Δ (mm.s ⁻¹)	Γ_{exp} (mm.s ⁻¹)
A ₁	- 0.052 3 ± 0.005 8 0.685 ± 0.005 9	1.754 ± 0.018	0.316 ± 0.004 18	0.369 ± 0.048	0.483 ± 0.006
A ₂	0.445 ± 0.068 2	9.328 ± 0.069	0.445 ± 0.068	—	0.325 ± 0.041
B ₁	0.396 ± 0.045 2 2.854 ± 0.036 9 - 0.376 ± 0.068 5	0.223 ± 0.010 0.158 ± 0.084	1.625 ± 0.029 - 0.376 ± 0.068 5	1.329 ± 0.029	0.697 ± 0.052
B ₂	- 0.322 ± 0.042 0.652 ± 0.041 0	9.922 ± 0.091	0.160 ± 0.042 3	0.482 ± 0.031	0.500 ± 0.020
C ₁	- 7.03 ; - 5.21 ; - 3.32 ; 0.02 ; 0.723 ; 2.78 ; 4.51 ; 7.33	min. 0.33 max. 1.45			min. 0.53
C ₁	0.126 ± 0.031 2 0.514 ± 0.032 2	9.013 ± 0.034	0.194 ± 0.032 4	0.320 ± 0.032 4	0.324 ± 0.021

A-6-aminopurine-Fe ; B-adenosine-3'-phosphate-Fe ; C-guanosine-3-phosphate-Fe ;
 A₁, B₁, C₁-solution ; A₂, B₂, C₂-lyophilized sample.
 δ -isomer shift ; Δ -quadrupole splitting.

racter of Mössbauer spectra of lyophilized samples did not change compared to water solution complexes, where we have found changes of Mössbauer spectra in all of them. We have also seen the changes of isomeric shift in Mössbauer spectra, which are at the same time not dependent on the dose of UV-irradiation for all the samples. In all the cases of quadrupole doublet type we have found enlargement of the splitting of Mössbauer spectra.

The Γ_{exp} values in UV-irradiated samples are always lesser as compared with those of the native samples.

When assuming [1] the shift of absorption maximum in light spectrum of the complexes from the maximum values for the organic substance to the values of absorption maximum for FeCl₃.6 H₂O ; [2] the magnitude of quadrupole doublet splitting after UV-irradiation of all the samples and [3] the decline

of Γ_{exp} after UV-irradiation in the spectra of the complexes we can presume that in organo-metallic complexes, as seen on figure 1 the processes appeared after UV-irradiation take part mostly on iron ion side (in its near vicinity) and not on the side of the rest of organic substance molecule.

It is important to point out that the Mössbauer spectra of the complexes as measured after UV-irradiation cannot give us the precise state in the complex which is caused by the absorption of UV-irradiation. The Mössbauer spectra measurements, taking into account the small values of resonance effect, have continued for a period approximately of 20-30 hrs., that means the resulting parameters express only the mean values in time of the different states of the complex calculated as mean values for the whole period of Mössbauer spectra measurements.

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APPLICATION OF THE MÖSSBAUER EFFECT TO THE INVESTIGATION OF DYNAMIC STRUCTURE AND CATALYTIC PROPERTIES OF ENZYMES

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Résumé. — La mobilité locale de protéines a été étudiée à l'aide des marqueurs Mössbauer, spin ou proton. L'augmentation de la température des solutions de protéines et l'accroissement du degré d'hydratation d'échantillons anhydres diminuent le facteur f' des noyaux Mössbauer situés aux centres actifs ou liés à la matrice de protéine (α -chymotrypsine, séroalbumine). La diminution de f' est due apparemment à des mouvements spontanés de coupure des chaînes polypeptidiques par suite des fluctuations thermiques. On a trouvé une corrélation entre les propriétés dynamiques, le degré d'hydratation et l'activité catalytique de l' α -chymotrypsine. Le temps de corrélation des mouvements de coupure dans les protéines est de l'ordre de 10^{-11} à 10^{-7} s ainsi qu'on le déduit de la mobilité des marqueurs par RGN, RPE et RMP. Les modifications spontanées de conformation peuvent être des mécanismes d'adaptation dynamique et de transitions allostériques et transglobulaires.

Abstract. — The local mobility of the protein macromolecules has been studied by using the set of the Mössbauer, spin and proton labels. The increase in temperature of the protein solutions and the increase in hydration degree of dry samples result in the decrease in f' of the Mössbauer nuclei, entering the active centres or bounding with the protein matrix (α -chymotrypsin, serum albumin). The decrease in f' is accounted apparently by spontaneous segmental motions of polypeptide chains as result of heat fluctuations. The correlation between the dynamic properties, hydration degree and catalytic activity of α -chymotrypsin was found. The correlation time of segmental motions in the proteins is found in the region 10^{-11} - 10^{-7} s by analysis of mobility of the labels by means of gamma-resonance spectroscopy, ESR and PMR. The spontaneous conformational changes may provide mechanisms of the dynamic adaptation, allosteric and transglobular transitions.