УДК 577.3

МОЛЕКУЛЯРНА БІОФІЗИКА

DNA STRUCTURAL CHANGES DURING INTERACTION WITH METAL IONS ON IR- SPECTROSCOPY DATA

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Received on November 6, 1998.

The metal (Mn^{2+} ,Mg^{2+} ,Ca^{2+} ,Cu^{2+}) ion effect on the DNA structure in films is studied at different relative humidities (5-98%) by the IR-spectroscopy method. The results obtained evidence the interaction of the ions with DNA phosphate groups as well as with nucleic bases. The formation of the secondary structure of DNA complexed with metal ions is shown to take place at the greater number of water molecules bound to the polymer than in the case of DNA without ions. The interaction of DNA with metal ions prevents its transition into A-form and inducees essential changes in the hydrate water of the complexes. The models for the ion interaction with DNA macromolecules and its components are discussed.

Keywords: DNA structure, metal ions, vibration spectroscopy

The active biological role of divalent metal in the function of the genetic apparatus in cancerogenesis and mutagenesis has been attracting permanent interest in the interaction of these ions with nucleic acids. A great number of works has considered this problem [1-10] but at present binding sites of Me on DNA, molecular mechanisms of interactions of the hydrate water with DNA complexed with metal ions have not been clarified yet.

The present work continues the previous studies carried out using the viscosimetry, CD and differential UV-spectroscopy methods [8,9]. The work studies Mn^{2+} ,Ca^{2+} Mg^{2+} and Cu^{2+} ion interactions with DNA in films at various relative humidities by IR- spectroscopy. The method used gives information on metal ion binding sites, the metal ion effects on the macromolecule transition into the double helical conformation and on properties of the hydrate water of the complex. Such data are necessary to develop real models of the Me ion+DNA hydrate complex and to understand mechanisms of ion-hydrate environment effects on the structure and parameters of macromolecules.

EXPERIMENTAL

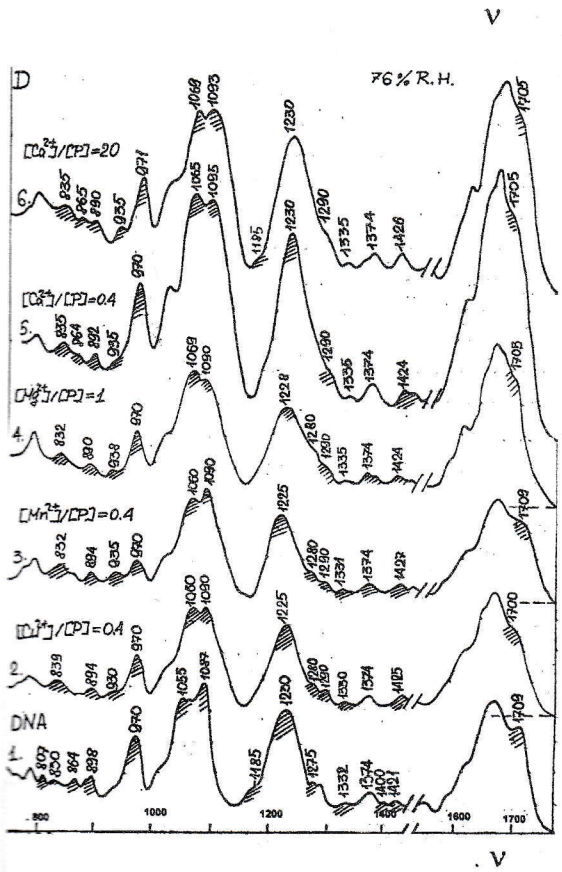
Native calf thymus DNA of molecular weight 1.9×10^7 Da was used, the protein content being smaller than 0.5%. DNA was extracted in the D.Yu. Lando Laboratory (Minsk, Belarus). The hypochromic effect was 36% at λ = 260 nm. The number of elements Na^+ and K^+ determined with a FPL-I flame photometer in DNA preparations (regarding their "dry" weight) was 7.0 ± 0.2 and 0.6 ± 0.02%, respectively.

Methods of the DNA film preparation and IR spectum recording with an IR-spectrophotometer UR-20 (Zeiss, Jena) are described in details elsewhere [4]. IR spectra of DNA complexes with the above ions were studied in the range of relative humidities (RH) from 5% to 98%. The temperature was 29°C. The average number of water moles per mole of DNA nucleotides *(n)* was determined as described in [4].

RESULTS AND DISCUSSION

To investigate Me^{2+} ion effects on the structure of DNA and its hydrate shell, IR spectra of DNA complexes with Mn^{2+} ,Ca^{2+} and Cu^{2+} ions were studied in the range of relative humidities (RH) from 5% to 98% (Figs.1,2). As binding constants for the Mn^{2+}, Mg^{2+} ,Ca^{2+} and Cu^{2+} ion association with DNA differ essentially, various concentrations of ions ($[Cu^{2+}]/[P]=0,4$, $[Mn^{2+}]/[P]=1$, $[Ca^{2+}]/[P]=10\text{-}20$) were studied in order to compare effects induced by Me^{2+} ions. The analysis of the spectra obtained shows that the above metal ions induced essential changes in IR-spectra of DNA in the absorption region of the sugar-phosphate bone as well as of the nucleic bases (Figs.1-3).

Thus, Ca^{2+} ions induce shifts and intensity changes of the absorption band of asymmetric (ν =1230 cm^{-1}) and symmetric (ν =1087 cm^{-1}) vibrations of PO_2^- groups and 14 cm^{-1} shift of the absorption band of ribose (ν =1055 cm^{-1}) in DNA at $[Ca^{2+}]/[P]=20$ and n=8 (76 % RH) (Fig. 2). At



. Fig 1. IR spectra of DNA+Me^{2+} complexes at 76% RH.

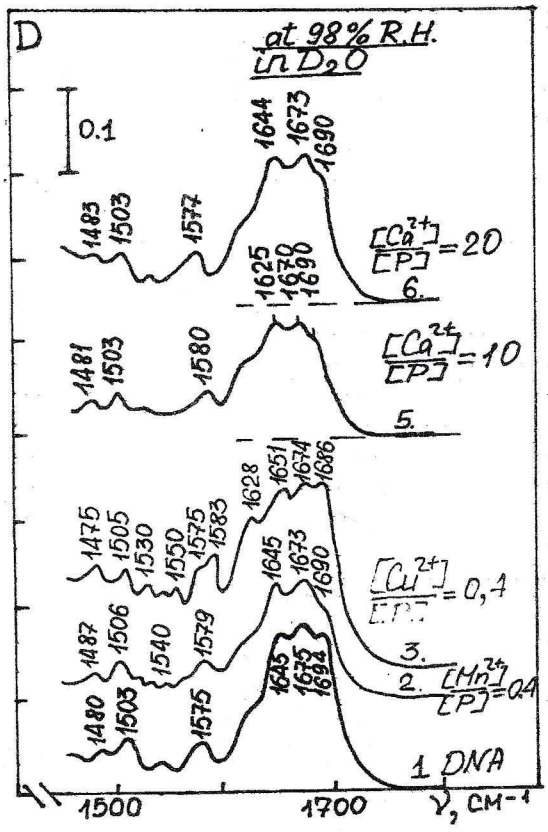


Fig 2. IR spectra of DNA+Me^{2+} complexes at 76% RH in D_2O.

n>12 (98 % RH) these shifts of 1221 cm^{-1} and 1087 cm^{-1} absorption bands decrease, being 1 - 3 cm^{-1}. Also, Mn^{2+} ions cause shifts of absorption bands of asymmetrical vibrations of PO_2^- groups by 5 cm^{-1} to the region of low frequencies ([Mn^{2+}]/[P]=0.4) at 76 % R.H. (Fig. 1). With the increase of the water content in the film up to n >16 the absorption band of phosphates (ν =1221cm^{-1}) shifts up to ν =1222 cm^{-1}. Cu^{2+} ions induce shifts of phosphate absorption bands at n=8. For example, the absorption band of asymmetrical vibrations of phosphates for DNA+Cu^{2+} is 1225 cm^{-1}.

Table 1 gives main characteristics of absorption bands of DNA and DNA complexed with Me^{2+} ions. These data and spectra in figs. 1-3 evidence that Mn^{2+}, Ca^{2+} and Cu^{2+} interact with DNA phosphates and that DNA complexed with divalent ions seems to transit into B-form passing A-form. Changes in parameters of spectra of deuterized DNA films and DNA+Me^{2+} ion complexes are shown in Table 2 It should be noted that the absence of the band 1710 cm^{-1} and the shift of the band 1053 cm^{-1} characteristic of the presence of the double-helical DNA structure, in addition to the location of absorption bands of PO_2^- groups, do not permit the conclusion on the formation of the native DNA structure complexed with Ca^{2+} ions (Ca/P=0,4÷1), Mg (Mg^{2+}/P=1) even at R.H.=98% (Fig. 1-3, Table 1). The double-helical structure is absent with the all DNA metal complexes studied at R.H.=76% (Fig. 2). Its formation completes at R.H. 98%. DNA films kept in a hermetic cuvette with H_2O or D_2O for several (2-3) days showed that metal complexes sorb the larger number of water molecules (n) than the pure DNA and that this number increases with the rise of the Me^{2+} content in DNA films [4,5]. So, for DNA+Ca^{2+} at Ca^{2+}/P=20 n were 26 while for DNA it was 20 and did not increase on the DNA keeping in a hermetic cuvette with water for 2-3 days.

Analysis of changes in spectral parameters of DNA (Figs 1) and deuterized films of DNA+Me^{2+} complexes (Fig. 3) is given in Tables 1 and 2 , respectively. The comparison of frequencies of characteristic absorption bands of IR spectra of DNA and its complexes with Me^{2+} ions permits a supposition that in the presence of Me^{2+} studied DNA transits into the double-helical conformation at higher R.H.s than the pure DNA. Unlike the latter, the above DNA seems to transit to the B-form

Table 1 **Main marker bands in IR spectra of complexes DNA+Me^{2+}.**

DNA A-form	exp.DNA 76% RH	DNA+Cu [Cu]/[P] =0.4	DNA+Mn [Mn]/[P] =0.4	DNA+Ca [Ca]/[P] =0.4	DNA+Ca [Ca]/[P] =20	DNA B-form [8]	exp.DNA 98% RH	DNA+Cu [Cu]/[P] =0.4	DNA+Mn [Mn]/[P] =0.4	DNA+Ca [Ca]/[P] =0.4	DNA+Ca [Ca]/[P] =20	Assignment **[11-14,16-18]**
						1714	1715		1712	1715		B-f Base double bond
1709	1709	1700	1709	1705	1705			1710			1709	A-f inplane str. mode
1695	1695	1690	1688			1694	1694	1686	1690		1690	C=O (T), C6=O (G)
1670	1670	1660	1665		1662	1675	1675		1673		1673	C6=O(G), C=O(T)
1620	1620	1630	1625			1620	1620	1628			1625	C=N (A,G)
1575	1575	1575	1578		1580	1575	1575		1579		1580	C=N (G)
		1425	1427	1424	1426	1425	1422	1425	1426	1429	1429	C2' endo
1421	1419										1419*	C3' endo
1374	1374	1374	1374	1374	1374	1374	1374	1374	1374	1374	1374	dG, dA anti
						1327	1329	1329	1328	1329	1329	B-form T
1331	1332	1330	1331	1335	1335							A-form dA+dT
		1280	1280*	1290	1290*	1281	1281	1290	1280	1285*	1290	B-form T
1277	1275											A-form T
1234	1230			1230	1230							A Antisym. phosph.
		1225	1225			1223	1224	1216	1222	1225	1227	B stretch. vibration
1189	1185*				1185*						1185*	A furanose
		1060	1060	1065	1069							furanose
1053	1055						1055	1058	1055	1058	1058	C-O, anti
968	970	970	970	970	971	969	970	971	971	970	970	
		930	935	935	935	936	940	931	938	938	940	B marker
		894	894	892	890	894	894	896	894	894	894	B marker
897	898									898*	898*	A marker
885	888											A marker
864	864			864	865					865*	865	dC3' endo
	830*	839	832	835	835	833	841	840	840	837	837	d C2' endo
807	807										809*	A marker

* - shoulder

conformation family, passing the A-form (Table 1). Besides, we note strong conformational changes in DNA+Cu^{2+} and a DNA disordering at Cu/P≥0,4. Data in Table 2 permit analysis of the shift of characteristic absorption bands of DNA nucleic bases on the DNA interaction with Me^{2+}. Besides, they permit a supposition that the interaction of Mg^{2+}, Ca^{2+} and Mn^{2+} ions with DNA is realized by coordination with N7 and O6 of guanine and phosphate groups. For Cu^{2+} ions this complex can be supposed only with the ion content smaller than $Cu^{2+}/P<0,4$. In the case of high concentrations of $Cu^{2=}$ ions a model proposed in [10] seems to be realized. In this model Cu^{2+} ions coordinate with N7 and O6 of guanine and N3 and O2 of cytosine, locating inside the DNA double helix[5]. Such a complex is possible in the case of the guanosine rotation around the glycoside bond and the transition to sin-conformation that, as studies showed, is realized on DNA protonation [8]. Studies on DNA+Cu^{2+} complexes was carried out by Raman spectroscopy [5,9] evidence as well the realization of sin-conformation on the Cu^{2+} binding to DNA at $Cu^{2+}/P>0,4$.

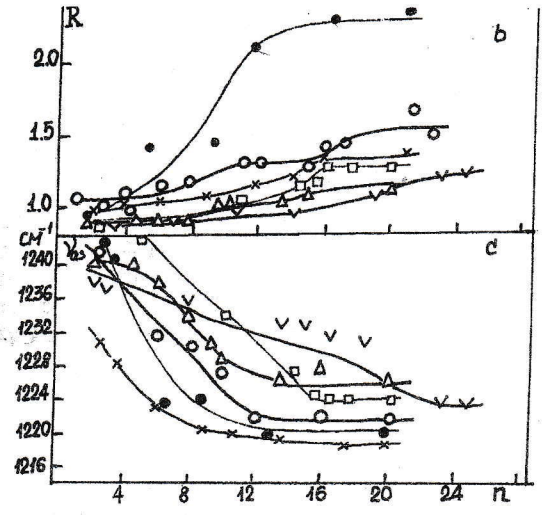
Table 2 Parameters of spectra of deuterized DNA films and DNA+Me^{2+} ion complexes

ions / R.H.	DNA with Mn^{2+} 1:0,4	DNA with Cu^{2+} 1:0,4	DNAwithCa^{2+} 1:20	Assignments [SemenovM; Ghomi M. et al; Fritzsche H.et al]
76%	1575→1578	(N) 1550 1575↑	1575→1580	C=N(C)at presentCu^{2+} C=N(G)
	1620→1625	(N) 1580 1620→1630		C=N(G)at presentCu^{2+} C=N(C,A)
	1670→1665	1645 →1660↑	1645↑	C_2=O(C)
	1695→1688↑	1670 1695→1690↓	1670→1662	C_6=O(G), C=O(T) C=O(T), C_6=O(G)
98%	1575→1579	(N)1550	1575→1580	C=N(C)at presentCu^{2+} C=N(G)
	1675→1673	(N)1583 1620→1628↓	1620→1625	C=N(G)at presentCu^{2+} C=N(C,A)
	1694→1690↓	1694→1686↑	1675→1673 1694→1690↓	C_6=O(G), C=O(T) C=O(T), C_6=O(G)

↑↓- decrease or increase of the absorption band intensity
→ - shift of absorption bands in spectra of DNA+Me^{2+}complexes are indicated in comparison with DNA spectrum;
(N)-new absorption band.

The analysis of dependencies of frequencies and intensities of absorption bands of nucleic bases and the sugar-phosphate bone on the number of absorbed water molecules per nucleotide (n) allows to study interactions with ions and structural changes of macromolecules. The absorption band intensity was characterized in relative units $R = D_i/D_0$, where D_i and D_0 are optical densities at the maximum of absorption bands at i- and minimum relative humidities, respectively. The minimum relative humidity was 5%.

Figures 3 show dependencies of frequencies and intensities of stretching vibration bands of DNA phosphate groups complexed with divalent ions. It is known that for DNA such dependences permit to observe the transition into A- and B-form at n=8 and n=12, respectively [15]. It can be seen that for DNA+Me^{2+} ion complexes essential changes in the character of the above dependences are observed. Thus, Mn^{2+} and Ca^{2+} ions decrease the intensity of these DNA absorption bands, while Cu^{2+} ions increase it. Probably, the decrease of the intensity is due to the fact that Ca^{2+} and Mn^{2+} ions interacting with phosphates prevent from their hydration, while Cu^{2+} ions are able to locate inside the DNA helix, forming interstrand complexes [5]. Besides, they do not prevent from the hydration of the bone, and the increase of the intensity could result from P=O bond polarization during the interaction. Cu^{2+} ions with DNA



Figs.3. Dependencies R and ν values for PO^{-}_2 group vibrations on number of the sorbed water molecules (n) for DNA and DNA+Me^{+2} complexes:1-° DNA; 2-• Cu^{2+}/P=0.4; 3-× Mn^{2+}/P=0.4; 4-Δ Mg^{2+}/P=1; 5-□ Ca^{2+}/P=1; 6- ∨ Ca^{2+}/P=20

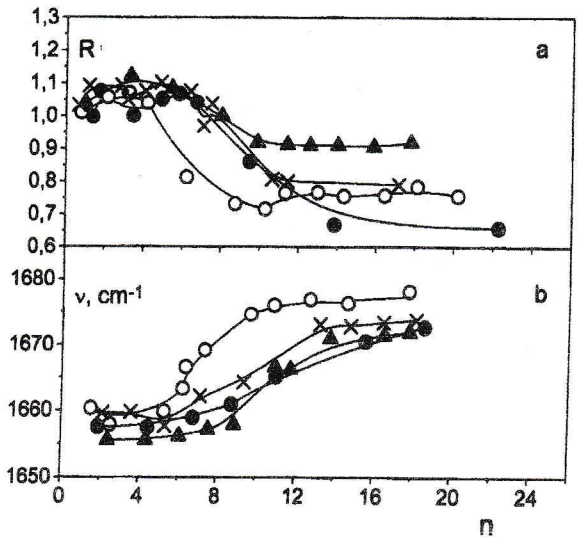


Fig.4. Dependencies of the frequency (ν) and relative intensity ($R=D_i/D_0$, where D_i and D_0 are the optical densities at the maximum of absorption band at i and minimum relative humidities, respectively) on the number of absorbed water molecules: (O)DNA, (×)DNA+0.4[Mn^{2+}]/[P], (●)DNA+0.4[Cu^{2+}]/[P], (▲)DNA+20[Ca^{2+}]/[P].

At n<3 frequencies of asymmetrical vibrations of phosphate groups of DNA+Me^{2+} ion complexes, except Cu^{2+} ions, are shifted to the region of lower frequencies as regards those in the DNA spectrum. Also, it should be noted that for DNA the transition into the double helical conformation (B-form) is identified by the shifts of phosphate absorption bands to ν =1224 cm^{-1}, 1087 cm^{-1} (it occurs at n=12 for DNA in the absence of Me^{2+} ions [15]), while these bands are observed at n=18 for [Ca^{2+}]/[P]=10, n=24 for [Ca^{2+}]/[P]=20, n=14 for Mn^{2+} ions and n=12 for Cu^{2+} ions.

Data of Table 1 and dependencies obtained for intensities and frequencies of vibrations of phosphate groups of DNA complexed with Me^{2+} ions indicate that all the above ions delay DNA transition into the B-conformation. DNA macromolecules complexed with Me^{2+} ions transit into the B-conformation at n=14 for Mn^{2+} ions, n=18 and 24 for [Ca^{2+}]/[P]=10, 20, respectively, and n=12 for Cu^{2+} ions (Fig. 3). This effect was also observed for DNA complexed with monovalent ions [7].

Also, DNA transition into the double helical conformation is identified by the appearance of absorption bands (ν =1053 cm^{-1}) of ribose and the in-plane stretching vibration mode (ν= 1705 cm^{-1}) [14,15] in the IR spectrum of native DNA at n>8 (Table 1) The observed IR band with a peak position at ν=1705 cm^{-1} is fairly well reproduced by calculations with the double-strand structure of Poly U (1707 cm^{-1} for C =O(U) absorption band of IR spectrum) [15]. Divalent metal ions induce the low frequency shift of this absorption band in IR spectra. For DNA+Me^{2+} complexes the appearance of absorption bands at the above frequencies is observed at n=14 ([Mn^{2+}]/[P]=0,4; 1, [Ca^{2+}]/[P]=10), n=20 ([Ca^{2+}]/[P]=20) and n=12 in the case of Cu^{2+} ions.

At n<12 the absorption band of ribose is resolved at ν =1065-1070 cm^{-1} in the IR spectrum of DNA complexes with Ca^{2+} and Mn^{2+} ions. This band is not observed in the case of Cu^{2+} ions.

Dependencies of frequencies and intensities of absorption bands of DNA nucleic bases and DNA+Me^{2+} complexes on the number of absorbed water molecules show the delay of the DNA+Me^{2+} ion complex transition into the helical conformation and the above frequency shifts too (Fig. 4).

It should be noted that the decrease of the intensity of absorption bands of nucleic bases due to the DNA+Me^{2+} ion transition into the double helical conformation is observed at n=10 (n=8 for native DNA) (Fig. 4). The discrepancy of intervals for n values at which the structural transition of DNA+Me^{2+} ions is identified by dependencies of frequencies and intensities of absorption bands of phosphate groups and nucleic bases can be explained by the preferential binding of Ca^{2+} and Mn^{2+} ions to biopolymer phosphates and by hydration of these centres. Such a discrepancy is not observed in the case of Cu^{2+} ions, which is due to higher values of binding constants for these ions bound to DNA bases than those for phosphates [5,14]. All the divalent ions, except Cu^{2+} ones, induce the shift of the frequency of the absorption band of stretching vibrations of the absorbed water from 3420 to 3400

cm^{-1} , perhaps, due to the Ca^{2+} and Mn^{2+} ion interaction with H_2O in outer layers of the DNA hydrate shell (Fig. 5).

It is also known that the frequency shift of this absorption band is proportional to the excess enthalpy of water molecule evaporation from the complex [13,15]. This leads to a conclusion that the binding of water molecules to the DNA+Me^{2+} complex is stronger than that to DNA macromolecules without ions.

Also, it should be noted that on the complex formation the maximum number of absorbed water molecules in the DNA hydrate shell increases (Fig.3). It follows from the fact that on the long-term (for about 5 days) keeping of DNA+Me^{2+} films at R.H.=100% maximum numbers of absorbed water molecules for Ca^{2+} ions are 23 at $[Ca^{2+}]/[P]=10$ and 26 at $[Ca^{2+}]/[P]=20$. For Mn^{2+} and Cu^{2+} ions this value is 22.

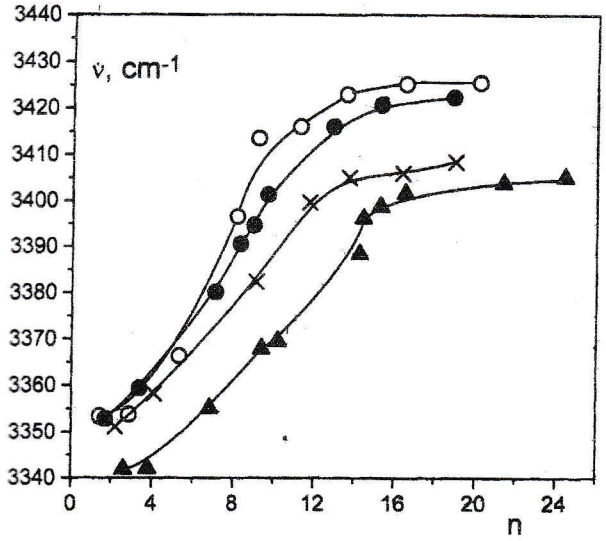


Fig.5. Dependecies of the frequency of the absorption band of absorbed water molecules on DNA: (O)DNA, (●)DNA+0.4$[Cu^{2+}]/[P]$, (×)DNA+0.4$[Mn^{2+}]/[P]$, (▲)DNA+20$[Ca^{2+}]/[P]$.

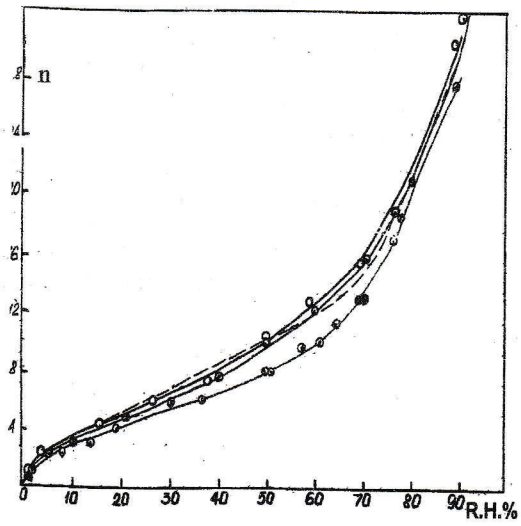


Fig 6. Dependence of sorbed water molecules (n) on relative humidity (RH) for DNA (1-°) and complexes with Cu^{2+} (2-◒) and Mn^{2+} (3-⊙)

The dependence of n on relative humidity of DNA + Cu^{2+} and DNA+Mn^{2+} by data of IR-spectroscopy and piesogravimetry methods are shown on fig. 6.

Binding DNA with Cu^{2+} decrease of the DNA sorption degree and RH =30-70%. In the range of RH=50-70% where DNA transition into double helix conformation is observed, the decrease of the DNA sorption degree is two water molecules per nucleotide (n=2). For Mn^{2+} ions this values $[Mn^{2+}]/[P]$ =0.4 is about n=1 and coincide with curve for DNA molecules without ions (Fig. 6). This effect can be explained by the fact that Me^{2+} bind with N_7 of the guanine and N_3 of the cytosine, which involved in hydration bonds formation. The DNA double helix structure formation is occurs at interaction water molecules with this centres on DNA molecules and dehydration N_7 of the guanine and N_3 of the cytosine during binding with the Cu^{2+} ions and in this case the number of the water molecules need for transition DNA + Cu^{2+} are increase .

Conclusion

The results obtained show that in the presence of metal ions DNA transits into the B form by passing the A form. All the investigated ions delay the DNA transition into the B conformation. The transition occurs at n = 14 for Mn^{2+} ions, n = 18 and 24 for $[Ca^{2+}]/[P]$ = 10 and 20, respectively, and n = 12 for Cu^{2+} ions. Cu^{2+} and Mn^{2+} ions decrease the DNA hydration while Ca^{2+} ions increase it. From the fact that shifts of frequencies of stretching vibrations of the water sorbed on the DNA+Me^{2+} complex are observed, it may be concluded that the binding of water molecules to a DNA + Me^{2+} complex is stronger than that to DNA macromolecules without ions.

The results obtained evidence the modification of the secondary DNA structure, specifically, the B-A structural changes in some part of the DNA molecule complexed with Ca^{2+} at high concentrations of calcium ions ($[Ca^{2+}]/[P]$ = 20). Studies of DNA + Cu^{2+} complexes ($[Cu^{2+}]/[P]$ = 0.4-0.6) show that the DNA structure cannot be assigned to any of the known conformations (A,B,Z). The purine nucleotide transition from anti to syn conformation was observed in DNA complexes with Cu^{2+} ions and at the high ($[Ca^{2+}]/[P]=20$) concentration of Ca^{2+} ions. These data can support the model of the DNA + Cu^{2+} complex proposed in Refs. [9,10].

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