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REACTION OF NUCLEIC ACIDS WITH METALS

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1. INTRODUCTION

Nucleic acids play an important part in the processes of the biosynthesis of protein, growth, reproduction, and heredity of living organisms. However, even now very little is known about the actual mechanism of their participation in these processes; this explains the considerable interest of investigators in the various types of reaction of nucleic acids.

In recent years it has been found that nucleic acids react with ions of many metals, the result being a change in several properties of the acids. It is extremely probable that the reaction with metals is of great importance in the biological functions of nucleic acids but there have been very few investigations in this direction and there is still no direct proof that metals play an essential part in the biological functions of the nucleic acids.

The study of the reactions of complex formation between metals and nucleic acids is also of independent interest in connection with the search for so-called electronic stains for electron microscopy of nucleic acids (metal ions in compounds with the nucleic acids can act as electron dyestuffs) and for obtaining especially pure preparations of nucleic acids.

In order to understand the reactions which take place between metals ions and nucleic acids, we will briefly consider the structure and properties of the latter.

2. STRUCTURE AND PROPERTIES OF NUCLEIC ACIDS

The nucleic acids are linear polymers [1] the chains of which are composed of residues of phosphoric acid, pentoses [deoxyriboses in deoxyribonucleic acid (DNA) and riboses in ribonucleic acid (RNA)] and heterocyclic nitrogen bases (purines and pyrimidines).

Sections of the polymeric chains of DNA and RNA are shown in Fig. 1, which represents conventionally four links in each chain. The monomer links differ from one another in the structure of the purine or pyrimidine bases. The natural long molecules of nucleic acids consist of many thousand differently alternating monomeric links. It is evident from Fig. 1 that the DNA chain differs from the RNA chain not only in the composition of the sugars but also in the composition of the pyrimidine bases. The thymine present in DNA is replaced by uracil in RNA.

X-Ray diffraction study of films of nucleic acids showed that the DNA structure is much more ordered than the RNA structure, and that DNA is a two-chain spiral, diameter 20 Å and length of turn 34.6 Å. A turn of the spiral contains ten monomeric links. The lateral groups — purine and pyrimidine bases — are packed in the centre of the spirals.

According to the model of Crick and Watson[2], these bases in the two-chain DNA molecule are located in such a manner that the adenine in one chain is always opposite the thymine in the other, and the guanine is opposite the cytosine. The two chains are connected with one another by hydrogen bonds (see Fig. 2).

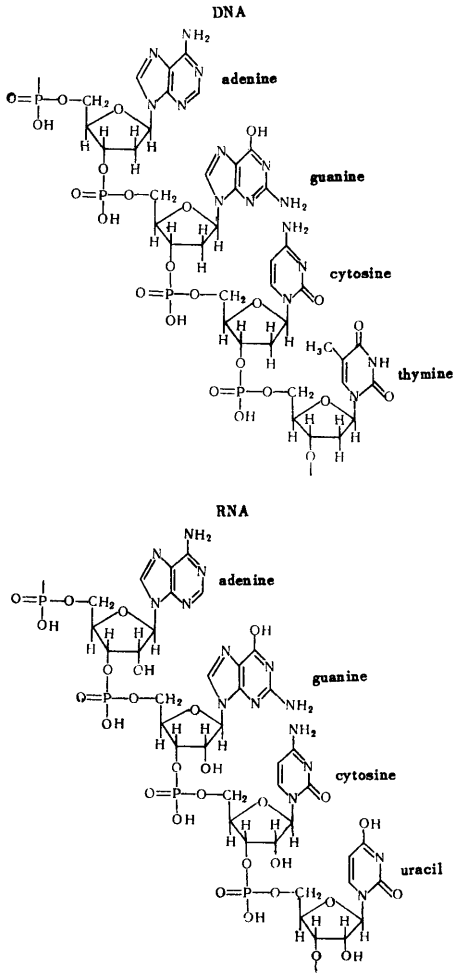


Fig. 1. Sections of the polymeric chains of DNA and RNA.

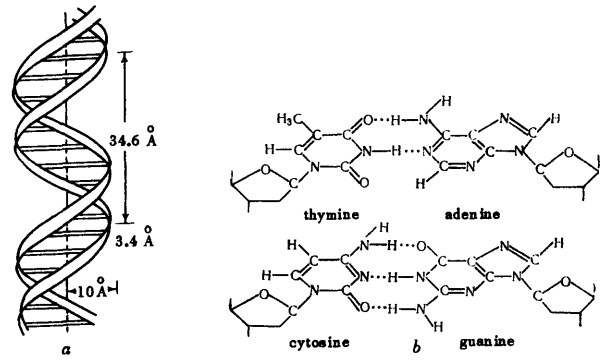


Fig. 2. Macromolecular structure of DNA: *a*) two spirals: phosphato-deoxyribose chains; horizontal lines: pairs of nitrogenous bases connecting the two chains by hydrogen bonds; *b*) hydrogen bonds in pairs of bases.

The RNA molecule consists of one flexible polynucleotide chain, the configuration of which is not stable and fixed, but is determined by the surrounding medium[3-6]. Thanks to the hydrogen bonds, in some places the RNA chain is turned into a double filament, formed by separate turns of the spiral. Between the turns, single-chain sections are situated randomly. This secondary structure of RNA in turn does not remain stable — depending on the conditions of distribution of the spiral portions it can become ordered: more complex compact tangles of tertiary structure are formed. Fig. 3 shows the macromolecular structure of RNA[5].

The hydrogen bonds create a definite structural rigidity in nucleic acids, which is evidently a general characteristic of the macromolecules of biopolymers and determines their biological function[7]. The destruction of the hydrogen bonds of isolated nucleic acids is observed over a narrow range of temperatures, in which "fusion" of the intramolecular regular structure occurs with change of the spirals into disordered tangles[8]. This process is termed denaturing. The change from the native stage into the denatured ("phase transformation") can occur not only on heating,

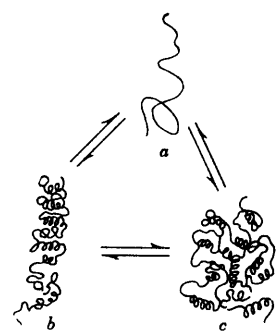


Fig. 3. Macromolecular structure of RNA: *a*) primary structure — loose, random tangle or straightening chain; *b*) secondary structure — compact rod with more or less ordered orientation of the double-spiral portions; *c*) tertiary structure — compact tangle with spiral portions.

but also as the result of other factors. This transformation can be followed by the change in light absorption of solutions of nucleic acids in the ultraviolet region.

In the spectrum of the nucleic acids there is an absorption band in the near ultraviolet region, which is due to light absorption by the chromophoric groups of the heterocyclic bases. The maximum of the band is approximately at 260 mμ. The optical density of native nucleic acids is ~ 40% less than the total optical density of their monomer constituents. This fall (hypochromic effect) is explained by the parallel arrangement of the chromophoric groups of the bases in the native preparations. The optical density rises during denaturing. This hyperchromic effect is explained by the destruction of the parallel arrangement of the chromophoric groups of the bases during the phase transformations from the spiral-shaped ordered forms to the less ordered. The decrease in optical density thus shows the increase in the structural rigidity of nucleic acids due to the increase in spirilisation, but an increase in optical density shows their degradation into disorientated chromophoric groups, in any breakdown of ordering [1,9-12].

Some information about the dimensions, form, and molecular weight of the nucleic acid macromolecules was obtained by sedimentation analysis and measurements of viscosity and diffusion rates [13].

3. EFFECT OF METAL IONS ON THE PROPERTIES OF NUCLEIC ACIDS

The nucleic acids isolated from different biological objects usually contain insignificant amounts of ions of one or more metals [14-28]. Thus Wacker and Vallee [29] reported the presence of chromium, nickel, iron, manganese, zinc, and other metals in preparations of DNA and RNA. The concentration of some metals in nucleic acids of different origins is constant, but the bond between the metals and nucleic acid is so strong that they are removed with difficulty even using such strong chelating reagents as ethylenediaminetetraacetic acid, quinolin-8-ol, dithizone, etc.[29] These facts are evidence that the metals form chemical bonds with the nucleic acid macromolecules.

It was stated above that there are still no direct proofs of the participation of metals in the biological function of the nucleic acids. However, it is suggested that the metal ions bring about a bond between the nucleic acids and proteins [30], play a fundamental part in the structure and function of nucleoproteins [31,32], and affect the synthesis of proteins and the transmission of heredity information [29]. Gol'dshtein and Gerasimova [33] think that Fe^{3+} ions have a special role in the function of DNA: they take part in the formation of bonds between separate chains of DNA and as a result are able to regulate the denaturing of DNA, and are also the cause of the enzymeless transverse cleavage of the DNA molecule. According to the hypothesis of Ivanov [34] the biosynthesis of DNA is linked with the redox changes of iron or copper ions during their chelation with DNA, in which the oxidation of the metal ions brings about a weakening of the bonds between the complementary pairs of DNA bases, and reduction the stabilisation of the double-track conformation of DNA. Ascorbic acid, which participates in the synthesis of DNA in the cell, is a possible electron carrier in the oxidation and reduction of iron in DNA [35]. The correctness of this type of hypothesis is confirmed by the change in the properties of isolated nucleic acids and nucleoproteins under the action of ions of various metals. We will list some of these factors.

The dimensions and form of nucleoproteins suspended in cytoplasm and the degree of their polymerisation depends on the concentration of magnesium ions in the medium [32,36-38]. Alkaline earth metal ions affect the structure and properties of chromosomes [39-44]. In the presence of metal ions RNA is stable in relation to the action of ribonuclease [45,46], the macromolecular configuration of RNA depends on the ionic strength of the solution [6,12]. Various metal ions change the infectivity of tobacco mosaic virus RNA [25,47]. For example, it is found that the infectivity of the RNA of this virus correlates with the iron concentration in the preparations [25]. Nickel ions affect the infectivity of RNA more strongly than any of the other metal ions [47]. True, some authors [48] suggest that metal ions affect only the physical properties of tobacco mosaic virus RNA and not alter its infectivity.

The physical properties of RNA depend considerably on the presence of metal ions. Thus the sedimentation rate of solutions of nucleic acids [49] and their viscosity [50] decrease in the presence of Mn^{2+}, Ni^{2+}, Ca^{2+}, and Mg^{2+} ions. Na^{+} and K^{+} ions have a much weaker action than these and to bring about the same effect the concentration of alkali metal ions must be 2–3 orders of magnitude greater than the concentration of bivalent ions.

The melting point of the nucleic acids depends on the ionic surroundings [3,51,52]. On adding sodium chloride to DNA there is a linear relation between the melting point and the logarithm of the ionic strength of the solution. The increase in melting point with ionic strength is explained [3,51] by the stabilisation of the DNA with respect to the denaturing. It is suggested that the addition of electrolyte leads to screening of the charges in the DNA chains and this decreases the electrostatic repulsion forces between the charged phosphate groups, facilitating an increase in the rigidity of the structure.

Manganese, cobalt, zinc, and nickel behave like sodium chloride in raising the melting point of DNA, but copper, cadmium, and lead ions depress it [53,54]. Under conditions which facilitate the destruction of hydrogen bonds (heating the solutions of nucleic acids to 80° or dissolving them in 6 M urea — a reagent capable of destroying hydrogen bonds) such metal ions as Ni^{2+}, Mn^{2+}, Zn^{2+}, and Cr^{3+} decrease the optical density of RNA (raise the melting point), but alkali and alkaline earth metal ions hardly affect it. The conclusion was [7] that ions of the transition metals of the first period stabilise the structure of nucleic acids due to the formation of additional bonds between the metal and the bases of the acid. The intramolecular bonds formed with the metal are strong enough to withstand the destructive influence of a rise in temperature or the action of a concentrated solution of urea. With RNA the stabilising influence of the metal ions appears only on heating, when the hydrogen bonds are destroyed.

The change in the properties of the nucleic acids during the action on them of various metal ions is reversible. Katz [21] first drew attention to this ability by the reaction of DNA with mercury. He found that during reaction with $HgCl_2$ the DNA molecules become much smaller, but are restored by the action of reagents which bind mercury. On adding mercury salts to DNA there was a decrease in the characteristic viscosity of the DNA solution and in the spectral shift [20,21,55]. In combining with mercury the chacteristic viscosity and the position of the maximum in the DNA absorption spectrum acquired their original values. Reversible changes were also observed in studying sedimentation rate, scattering of light, optical rotatory

dispersion[56], and also the transforming ability[57] of DNA preparations†.

Hence no doubts remain about the complete reversibility of the reaction of DNA with mercury. This was confirmed by studying this reaction by methods sensitive with respect to the form and dimensions of DNA (viscosity, sedimentation rate, scattering of light), the peculiarities of the stereochemical configuration (optical rotatory dispersion), the presence of chromophoric groups and their relative orientation (absorption spectra in the near ultraviolet region). This was also confirmed by biological methods (transforming ability).

Such reversibility is characteristic for the reaction between tobacco mosaic virus RNA and mercury(II) chloride[56]. Unlike DNA, however, here the biological changes are not completely reversible. Thus Hg^{2+} ions completely deactivate the infectivity of tobacco mosaic virus RNA, but removal of these ions does not lead to complete reduction of the infectivity of RNA in comparison with the control.

Evidently the reaction DNA with Fe^{3+} ions, which was investigated by Gol'dshtein and Gerasimova[33,58], belongs to this type of reversible reaction. On introducing sodium pyrophosphate, which can bind iron, into a solution containing DNA and Fe^{3+} ions the ability of DNA to hydrolyse decreases.

The study of such reversible reactions, which are accompanied by a profound change in the physical properties of the nucleic acids, can evidently be of great importance in explaining the biological functions of the nucleic acids.

4. COMPOUNDS OF NUCLEIC ACIDS WITH METALS

Only a few compounds of the nucleic acids with metals have been isolated in the individual state. These compounds have mainly been investigated with the aim of finding methods for purifying DNA preparations. We will examine the method of obtaining them in somewhat greater detail.

As long ago as 1924 Hammarsten[59] observed that salts of lanthanum and iron(III) precipitate DNA from solution. The precipitation is maximal at pH 2.5–4. It was shown that the lanthanum nucleate compound has a very low solubility. This observation was confirmed later[60,61]. Stern and Steinberg[62] isolated and analysed compounds of DNA with lanthanum, neodymium, praseodymium, samarium, and yttrium, by adding the metal chloride solution to DNA solution, filtering off the fibrous precipitate, and thoroughly washing it with water, then alcohol, and drying in air. The dry products resembled asbestos fibres and had the colour characteristic of the rare-earth element. Suspensions of these compounds in glycerol had a marked absorption of light in the visible region of the spectrum. For the neodymium nucleato-complex the maxima of the absorption bands were at 510–512, 520–525, 570–590 mμ, and those for the praseodymium nucleato-complex were at 440–450, 465–470, and 475–480 mμ. The bands were clearly displaced relative to the bands for solutions of the pure rare-earth chlorides. The nucleate bands were much broader and less sharp than those for the chlorides. The solubility of the compounds was very low; for example, in the filtrate after separation of the neodymium nucleate from an aqueous suspension neodymium could not be detected spectrophotometrically by its absorption at 790 mμ or nucleic acid at 260 mμ. Ultimate analysis of the products showed that they contained 10.5 % neodymium and 11.1% lanthanum respectively.

The reaction between the lanthanide chlorides and sodium nucleate follows the equation[62]:

$$Ln^{3+} + 3\,DNA^- \rightleftarrows Ln(DNA)_3$$

where Ln is the lanthanide and DNA^- is the anion of sodium desoxyribonucleate.

The phosphate groups of the polynucleotide chain are ~ 6 Å from one another. The combination of one atom of rare-earth element with three phosphate residues is explained[62] by the formation of a sharply convolute polynucleotide tangle from one two-chain DNA spiral with the lanthanide ion in its centre. It was also suggested[62] that lanthanum ions can connect phosphate groups of neighbouring nucleotide chains, forming a network of filaments of different DNA molecules cemented to one another. The concentration of the lanthanide in such a complex should be 14%, but, as mentioned above for lanthanum and neodymium, the lanthanide concentration found did not exceed 11%. This is still unexplained.

The stoichiometry of the complex formation of metals with DNA was determined conductimetrically[63,64]. On the conductimetric curves for the titration of DNA with magnesium, manganese, barium, and calcium ions there are breaks corresponding to the stoichiometric ratio of one equivalent of metal ion : one of nucleotide. The position of the breaks is independent of the degree of denaturing of DNA and of the initial concentrations of the solutions.

As, however, such a ratio is not always[65,66] attained, additional proofs of the simple stoichiometry of the complexes were made[67,68]. Conductimetric titrations were made of synthetic polynucleotides: polyadenylic (poly-A) and polyuridilic (poly-U) acids, which contain only one base. These acids are capable of reacting with one another (this ability depends greatly on the nature and concentration of the metal ions present in the solution[69-71]). As a result of the reaction, the two single-chain acids form the double-chain acid poly-AU, which has properties reminiscent of those of DNA[70,72,73]. It has been shown that the titration curves of the two polymers and poly-AU are similar and reminiscent of the titration curves of DNA with magnesium ions and with manganese ions. The break on the titration curves (when a large number of points was taken during the titration) corresponded to the addition of 1 equiv. cation per 1 mole polymer phosphate. This ratio was the same whether native or denaturated DNA was titrated[74].

The composition of complexes of uranyl with DNA was also determined graphically[75]. The equilibrium concentration of uranyl ions in the solution after precipitation of the uranyl–DNA complex was plotted on the graph against the total concentration of uranyl ion in the initial solution. The approximate composition of the complex was determined from the break on the curve and it was shown that the stoichiometry of the complexes formed changes with the pH and the uranyl ion concentration, as Table 1 shows[75].

† Transforming ability is the name given to the ability of DNA isolated from one strain of bacteria to transmit definite characteristics, distinctive to the given progeny, for example, resistance to a certain poison, to another strain of bacteria. Transforming activity is inherent only in the native form of DNA. This index can be used as an experimental quantitative characteristic of the depth of denaturing and renaturing of DNA (Ref.1, p.269).

TABLE 1. Stoichiometry of the complex formation of uranyl with DNA as a function of pH and uranyl ion concentration. The DNA concentration has been recalculated per nucleotide link or per phosphorus of the nucleotide.

Experiments made at constant DNA concentrations ($\sim 4-6 \times 10^{-4}$ M) and variable pH		Experiments at constant pH (~ 3.5) and variable uranyl ion concentrations	
pH	UO_2^{2+}/P molar ratio	UO_2^{2+} concentration, M	UO_2^{2+}/P molar ratio
2.3	0.34	$1 \cdot 10^{-2}$	0.7
3.6	0.49	$4.8 \cdot 10^{-2}$	1.3
3.7	0.53	$6 \cdot 10^{-2}$	1.5
4.8	1.72		
5.8	2.42		

Probably this behaviour can be explained by the ability of the metal to form a compound not with a definite functional group of the DNA but, depending on the conditions, with different groups: phosphate, amino-, and enol.

The change in the stoichiometry of the metal–nucleic acid complexes with the pH and metal concentration was also observed in studying complex formation of Co^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, and Mn^{2+} ions with RNA and DNA by the equilibrium dialysis and isotope dilution methods [76]. On increasing the metal ion concentration the bonding of metal increases. Table 2 gives the results of determining the value of the "degree of bonding" at different pH and constant metal ion concentration, expressed in the number of metal atoms per atom of DNA phosphorus.

The complex formation of different metals with DNA has also been studied by the NMR method [77]. The paramagnetic metal ions in aqueous solution shorten the time of spin-lattice relaxation of the water protons. The relaxation time for protons in aqueous solution containing paramagnetic metal ions depends not only on the metal concentration but also on its chemical environment. It is known that there is greater effectiveness in shortening the relaxation time when there are small ions or molecules in the environment than when the metal is inside a large molecule.

TABLE 2. Bonding of the doubly-charged ions of certain metals with DNA at various pH and constant metal ion concentrations. Concentrations: metal ion 10^{-2} g litre^{-1}; Ca^{2+} 2.1×10^{-2} g litre^{-1}; DNA 10^{-5} g.*

pH	No. of metal ions per RNA phosphorus atom					No. of metal ions per DNA phosphorus atom				
	Co^{2+}	Zn^{2+}	Ca^{2+}	Mn^{2+}	Mg^{2+}	Co^{2+}	Zn^{2+}	Ca^{2+}	Mn^{2+}	Mg^{2+}
3.6	0.140	0.056	0.058	0.035	0.032	0.028	0.020	0.029	0.018	0.029
4.0	0.144	0.078	0.068	0.042	0.045	0.036	0.026	0.040	0.026	0.035
4.4	0.137	0.090	0.062	0.049	0.045	0.037	0.028	0.043	0.026	0.038
4.8	0.129	0.095	0.059	0.047	0.051	0.039	0.029	0.043	0.024	0.035
5.2	0.115	0.088	0.065	0.047	0.048	0.040	0.026	0.042	0.022	0.031
5.6	0.110	0.081	0.059	0.047	0.046	0.037	0.024	0.041	0.019	0.030
6.0	0.114	0.092	0.067	0.050	0.052	0.040	0.026	0.040	0.022	0.030
6.4	0.114	0.086	0.066	0.050	0.048	0.042	0.028	0.039	0.020	0.029
6.8	0.102	0.088	0.067	0.051	0.043	0.037	0.027	0.040	0.020	—
7.2	0.104	0.085	0.069	0.050	—	0.031	0.028	0.042	0.021	—
7.6	—	0.089	0.068	0.048	0.045	—	—	0.036	0.021	0.029
8.0	0.109	—	0.071	—	0.044	0.038	0.029	0.039	—	0.031
8.4	—	—	0.071	—	—	—	—	—	—	—
8.8	—	—	0.071	—	—	—	—	—	—	—

* These concentrations do not agree with those given in Table II of Ref. 76 (Ed. of Translation).

TABLE 3. Determination of the number of DNA parts available for absorption.

Metal ion	No. of metal ions arriving per 1 mg DNA	No. of equivalents of metal arriving per nucleotide
Mn^{2+}	$7 \cdot 10^{17}$	0.7
Cr^{3+}	$7 \cdot 10^{17}$	1.1
Fe^{3+}	$8 \cdot 10^{17}$	1.2

A theoretical appraisal was made of the decrease in relaxation time during the bonding of a metal ion with one or another part of the DNA molecule (by the parts of the linking DNA we mean the phosphate, amino-, and enol groups of the molecule) and the calculated quantities were compared with the experimental results obtained. On this basis the probable parts of the bonding DNA with various metals were determined and the "capacity" of the DNA molecule in relation to these metals. Table 3 gives these quantities.

As Table 3 shows, the same ratio is retained for complex formation for ions of iron and chromium: 1 equivalent of metal to 1 of nucleotide. Manganese ions do not obey this rule.

In studying the reaction of metal ions with tobacco mosaic virus RNA using labelled atoms (the radioactivity of a solution of a salt of the metal before reaction with RNA was compared with the radioactivity of a mixture of solutions of the metal salt and RNA after removal of the metal–RNA complex from it by centrifuging), it was found that the RNA bonds 0.5 mole calcium salt per nucleotide and ~ 1 mole mercury or silver salt per nucleotide [78].

Despite the fact that the composition of the complexes formed by metals with nucleic acids often corresponds to simple stoichiometric ratios, it must be remembered that simple stoichiometry cannot be fulfilled on account of the possibility of complex formation by the metal with different functional groups of the nucleic acids.

5. STRUCTURE OF COMPLEXES OF METALS WITH NUCLEIC ACIDS

In studying the structure of compounds of the type mentioned the region of the metal–nucleic acid bond is usually determined. To identify the region, the reaction of the metals with native and denatured forms of DNA are often compared. When these reactions do not depend on the form of DNA, the bond between the acid and the metal is effected directly with the phosphate groups. When the reaction depends on the form of DNA, this is evidence of the formation of a bond between the metal and the purine or pyrimidine bases of the acid. In native DNA these bases, as already mentioned, are linked by hydrogen bonds, and consequently their reaction with metal ions is hampered

By use of conductimetric titration [63,64,74] it was found that magnesium, manganese, barium, and calcium ions behave similarly in relation to native and denatured DNA. The position and character of the breaks on titration curves of the acid by these ions are independent of the degree of denaturing and over a wide range of concentrations correspond to a definite stoichiometric ratio of DNA and metal ions. Consequently it can be assumed that these metal ions are connected with the phosphate groups of the DNA. This conclusion is also confirmed by the following facts [74].

It is known[79] that the dye Acridine Orange reacts only with the phosphate groups of nucleic acids in 1 : 1 ratio and that the conductimetric titration curves for DNA by this dye are analogous to the curves for the titration of DNA by Mg^{2+} or Ba^{2+} ions and also the curves for the titration of poly-A, poly-U, and poly-AU by these ions. Hence the reaction of these metal ions with the different polynucleotides is independent of the composition of the bases and is determined by the phosphate groups.

In studying the reaction of the same metal ions with nucleic acids by the equilibrium dialysis method[76] differences were also noted in the degrees of bonding for the same ion with different forms of DNA. The effect of magnesium and calcium ions on the fluorescence of the ring of adenine, adenosine, adenosine monophosphate, adenosine diphosphate, and adenosine triphosphate was also studied[80]. The maximal intensity of the fluorescence of these substances, observable at 395 mμ, changed in the sequence: adenosine < adenosine monophosphate < adenosine diphosphate, adenine < adenosine triphosphate. Magnesium and calcium ions lowered to an almost equal extent the fluorescence of adenosine triphosphate and adenosine diphosphate to the intensity of adenosine monophosphate and adenosine respectively. These results serve as yet another confirmation that magnesium and calcium ions are connected mainly with the phosphate moiety of the nucleotide and are not attached to the ring of the base.

Study of the effect of metal ions on the melting point of nucleic acids can also be used to determine the region of bonding. It is known[53,54] that Mn^{2+}, Co^{2+}, Zn^{2+}, and Ni^{2+} ions raise the melting point but Cu^{2+}, Cd^{2+}, and Pb^{2+} ions depress it. This is explained[53,54] by the fact that the metals of the first group are connected with the phosphate residues at the DNA surface, which prevents electrostatic repulsion of the negatively charged phosphate residues and stabilises the DNA molecule. The metals of the second of these groups are bound to the purine or pyrimidine bases inside the spiral, in the sites of the hydrogen bonds, and destabilise the DNA molecule.

By use of the NMR method[77] it was also found that Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Cr^{3+} ions are most probably bound to the phosphate groups and not the nitrogenous bases of the DNA, but Fe^{3+} ions are linked with the nitrogenous bases. As for copper ions, they bring about the aggregation of the DNA molecules and, it is thought[77], are connected with the outer phosphate parts of the DNA. However, in the EPR spectra of DNA solutions treated with Cu^{2+} ions signals are observed which are explained[81] by the formation of a bond between the copper ions and the bases of the DNA.

The reaction of uranyl ion with DNA[75] apparently occurs at the phosphate groups. In investigating the complex-forming ability of several phosphates in relation to the uranyl ion it was found that the molar ratio of uranyl to phosphorus increases with the number of phosphate groups in linear phosphate polymers. Undoubtedly DNA can be included among these polymers. Direct proof of the connection of the uranyl ion and the phosphate groups is given by the infrared absorption spectra of DNA solutions in the presence and absence of uranyl salts[75].

Fig. 4 shows the infrared absorption spectrum of DNA. The 900–1300 cm^{-1} band belongs[82] to the vibrations inside the phosphate groups and sugars, the strong 1500–1600 cm^{-1} band to the vibrations of the >C=N–, >C=O, and >C=C groups of the nitrogenous bases, and the 3000–3500 cm^{-1} band to the vibrations of the amino- and hydroxyl groups in the sites of the hydrogen bonds[83].

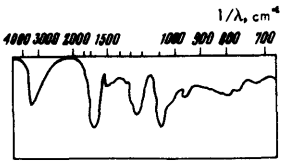


Fig. 4. Infrared spectrum of calf thymus DNA (sodium salt).

During complex formation with the uranyl ion some shifts in the position of the bands were observed only in the region from 900 to 1300 cm^{-1}. However, it must be noted that in comparing the infrared spectrum of DNA with that of the DNA–iron complex[84], only displacements of the bands of the stretching vibrations of the phosphate ester groups are observed and not a displacement in the region of the vibrations of the >C=N–, >C=O, and >C=C< groups of the bases. Moreover, it was found by differential spectrophotometry in the ultravoilet region (spectra of solutions of the metal–DNA complex recorded in relation to solutions of the free ligand and metal[85]) that iron ions also react with the DNA bases[84]. Similarly, in comparing the infrared spectra of adenosine triphosphate (ATP) and of copper–ATP complexes a displacement in the regions of the vibrations of the chromophoric groups of the bases was not observed. A displacement of the ATP absorption band into the ultraviolet region is observed in the differential spectrum and can be explained only by the reaction of copper ions with the bases[85]. The possibility of coordination between copper ions and purine components of the DNA has also been established in studying the effect of nucleic acids and their components on the oxidation of ascorbic acid, which is catalysed by copper ions[86].

Apparently infrared spectroscopy has still not unambiguously settled the problem of the locations of the bonds between the metals and nucleic acids.

Studying the absorption spectrum of DNA solutions in the near ultraviolet region in the presence of ions of various metals also allows conclusions to be drawn about the locations of the bonding of metals to DNA. It is known[87] that additions of Mg^{2+}, Ca^{2+}, and Mn^{2+} ions do not change the position of the bands in the DNA absorption spectrum, whereas Hg^{2+} or Ag^{+} ions displace the absorption band (Fig. 5)[55].

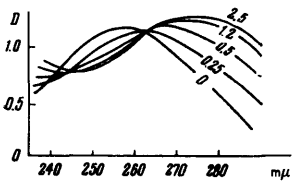
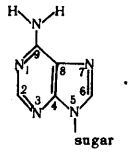


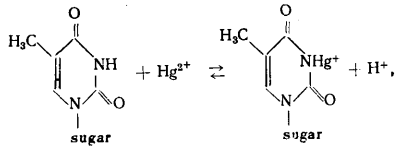
Fig. 5. Effect of $HgCl_2$ on the absorption spectrum of solutions of calf thymus DNA in 0.1 M $NaClO_4$, pH 5.6–5.7 at various ratios of moles $HgCl_2$ to moles DNA phosphorus (r). Values of r are shown on the curves.

In this connection it is suggested[55,88] that mercury and silver ions bond with DNA through the nitrogenous bases. Depending on the value of r (the ratio $[Hg^{2+}]/[PO_4^-]$) this reaction proceeds either with or without release of hydrogen ions[55,88]. This is explained by the attachment of the metal ions to different DNA bases, during which the hydrogen bond between the chains can be destroyed or remain unchanged.

When complex formation is not accompanied by the liberation of hydrogen ions it is suggested[55] that the metal ion is attached to the electron pair of the 7-N or 3-N of the purines adenosine or guanosine. The hydrogen bonds are not destroyed during this.

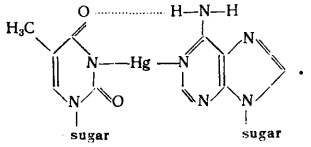


When hydrogen ions are released it can be assumed that there is a reaction of the type:



accompanied by the rupture of the hydrogen bonds.

Considering the reaction between mercuric ions and DNA, Katz[89,90] proposes that in the region $0 \leqslant r \leqslant 0.5$ compounds are formed in which the mercury play the part of an intermediate bridge between two coils of the DNA double spiral, the hydrogen bonds of which are not completely destroyed:



Such a complex is a two-filament polynucleotide in which almost all the bases of one filament are connected to the bases of the other by bridges of mercury atoms. The composition of such a complex corresponds to a mercury-nucleotide ratio of 1:2.

At $r \geqslant 0.5$ the filaments are separated, with formation of complexes of 1:1 composition by the reactions

$$\text{chain–base } a\text{–Hg}^{II}\text{–base } b\text{–chain} + \text{Hg}^{II} \rightleftharpoons \rightleftharpoons \text{chain–base } a\text{Hg}^{II} + \text{chain–base } b\text{Hg}^{II}.$$

A conclusion about the bonds between metals and the nucleic acid bases can be drawn from a study of the ultraviolet spectra only when there is a marked displacement of the maxima of the absorption bands of the nucleic acids as a result of the action of the metal, as happens for silver and mercury ions; when this is not so, it is necessary to study differential spectra in the ultraviolet region[85].

There are grounds for assuming that all the above mentioned methods of determining the structure of the metal–DNA complex do not give unambiguous results. It would appear, undoubtedly, that alkaline earth metal ions are bonded with DNA through phosphate groups. However, in using the equilibrium dialysis method[65] it was shown that Mg^{2+} ions act differently in relation to denatured and native forms of DNA. In this connection there is the possibility[54,91-93] that Mg^{2+} is bonded with the bases, mainly the purines, which have a structure reminiscent of quinolin-8-ol, whose great affinity for magnesium is well known.

It has been shown[93] that the addition of Mg^{2+} or Ca^{2+} to unionised nucleoside triphosphates brings about the same spectral shift as their neutralisation with OH^-. Probably, protons are displaced from the ring in both instances.

It is suggested[93] that because of the spiral configuration of the nucleoside triphosphate, the formation of bonds between magnesium and two parts of the nucleotide — the phosphate group and base — is possible.

Conformational analysis made with Steward models of atoms for the iron–nucleotide system[94] also confirm the possibility of bonds being formed simultaneously between metal, phosphate group, and base.

It is quite probable that the metal ions form bonds not with individual atoms of the rings of the bases but with the entire molecule of the base as a whole; this involves the overlap of the molecular orbitals of the base and the orbitals of the metal. It can be assumed that sometimes compounds are formed of the type of complexes with charge transfer using both the orbitals of the metal and molecular orbitals of the base and the d orbitals of the phosphorus atoms.

6. STABILITY OF COMPLEXES OF METALS WITH DNA

Some idea of the stability of the compounds formed can be obtained by comparing their association constants. Table 4 gives the values of the known association constants of complexes of DNA with various metals and shows that there are no sharp differences in the constants for different metals.

The example of silver complexes (Table 5) shows that the origin of the DNA has little effect on the association constants.

In calculating the constants it was assumed that the formation of a bond in each portion is independent of the occupation or unoccupation of the other parts. In one study[95] it was assumed that the composition of the complex corresponds to a metal:nucleotide ratio of 1:1. In the other studies the number of combining parts of the nucleic acid and the association constants were determined by a graphical method, the general principles of which for polyelectrolytes have been described[96].

TABLE 4. Association constants of complexes of metals with DNA.

Complex of DNA with the metal ion	Method of determining the equilibrium concentration of the metal	pH	Ionic strength	Association constant	Reference
Mg^{2+}	equilibrium dialysis		0.2	$2.8 \cdot 10^2$	65
Mg^{2+}	metal-indicator (Eriochrome T)	10	0.002	$2 \cdot 10^5$	97
UO_2^{2+}	titration with cyanoferrate(II)	3 5	0	$8 \cdot 10^6$	75
Ag^+	potentiometric	5.6	0.1	k_1 $2.4 \cdot 10^6$	88
Ag^+	potentiometric	5.6	0.1	k_2 $3.4 \cdot 10^5$	88
Fe^{3+}	kinetic	3.1	~0	$1.3 \cdot 10^5$	95

TABLE 5. Association constants of complexes of silver with DNA from various sources[88].

Specimen of DNA	k_1 $0<r<0.2$	k_2 $0.2<r<0.5$
Calf thymus DNA 1 native	$2.1\cdot10^6$	$4.1\cdot10^4$
Calf thymus DNA 2 native	$2.2\cdot10^6$	$7.4\cdot10^4$
Calf thymus DNA 2 denatured	$4.2\cdot10^6$	$1.3\cdot10^5$
E. Coli	$2.2\cdot10^6$	$5.7\cdot10^4$
M. Lysodeikticus	$3.1\cdot10^6$	$2.4\cdot10^5$

It has been shown[95] for the iron–DNA complex that the equilibrium constant is almost independent of temperature and consequently the heat of the reaction is small. On this basis the entropy of complex formation has been calculated to be ~ 23 e.u. The positive change in entropy is due to the dehydration of the ions during complex formation.

As stated above, metal ions, especially those of the transition metals, can be coordinated simultaneously with many functional groups, and for each group the stability constant will be different. Therefore association constants quoted are often mean values.

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For a long time it was thought that the occurrence of metals in nucleic acids was due to accidental contamination. From this review it is clear that metals can react with nucleic acids and the compounds formed often exhibit properties differing from those of the original acids.

One of the most interesting properties which characterise the complex formation between nucleic acids and metals is the reversibility of changes in the structure of the nucleic acids brought about by coordination with the metals. It is thought that the reversibility is a general feature of the behaviour of double-filament, polynucleotides consisting of two filaments bound by hydrogen bonds[98]. At high metal concentrations this reaction takes place up to complete separation of the filaments of the spiral of the DNA and the formation of 1 : 1 complexes. This form of separation of the chains is different from thermal separation in that chemical damage to the structural entity of the DNA (liberation of purines, breaking of phosphate ester bonds, etc.) is excluded completely.

Study of the absorption spectra of nucleic acids in the presence of $HgCl_2$ showed[56] that, in contrast to DNA, RNA forms a complex at $r > 1.0$. The same phenomenon was observed in experiments with DNA denatured by heating[55]. It is therefore proposed[56] that the reaction with $HgCl_2$ can also be used for finding differences between single-filament and two-filament polynucleotides.

Only a few metal ions (Hg^{2+}, Ag^+, and apparently Fe^{3+}) have yet been found which are capable of such reversible action towards nucleic acids. Probably a search for reversible reactions for other metal ions can give interesting results.

A necessary prerequisite for such investigations should be the determination of the regions of bonding of the metals with the nucleic acids. As the above review shows, there is no incontrovertible proof of a bond between the metal and any specified sections.

We see the importance of studying the complex formation of nucleic acids with metals also in the possibility of obtaining especially pure preparations of these substances. For this purpose quantitative characterisation of the complex formation is necessary — information about the association constants of the corresponding complexes. As we have seen, these data are extremely fragmentary. Moreover, we need further confirmation and reasons for the fact that the association constants for such different metals ions as Mg^{2+}, UO_2^{2+}, Ag^+, and Fe^{3+} are very similar.

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SPECTROSCOPIC STUDIES ON REVERSIBLE REACTIONS IN COMPOUNDS OF THE PORPHIN SERIES

A.N. Sidorov

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