ARCHIVES OF ACOUSTICS
11, 1, 89-96 (1986)

HYDRATION OF NUCLEIC ACIDS AND NUCLEOTIDES IN AQUEOUS ETHANOLIC SOLUTIONS DETERMINED BY ULTRASONIC METHOD*

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Hydration numbers of mono-and disodium salts of nucleotides as well as of sodium and potassium salts of *DNA* and *RNA* were determined from ultrasonic velocity measurements. For comparative purposes hydration numbers of monosodium and monopotassium phosphates were also determined.

1. Introduction

Hydration of nucleic acids is a subject of numerous experimental and theoretical investigations [1] – [22].

According to Jacobson [23] – [25] the structure of water surrounding macromolecules of DNA is similar in its order to that of ice. The double helix of DNA fits very well into the volume of empty cages in the quasicrystalline tetrahedral structure of water and being built into water lattice it stabilizes this structure. These long-range icelike structures around DNA, however, have not been confirmed by other investigations [7], [26]. NMR studies as well as dielectric constant measurements suggest rather local influence of DNA on the structure of water and bounding some amount of water in hydration sheath in which water molecules form a different structure and have different properties from those in the bulk. Another concept considers two- and three-state models of water. Despite richness of experimental and theoretical material reported so far, neither of the concept has been proved right. Both structure of aqueous sloutions of nucleic acids and the role of each

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* This work was partially financed by the Polish Academy of Science (Problem MR. I. 24).

fragment of polinucleotide chains in hydration of macromolecules of DNA have not been clarified yet.

Our studies on the structure of aqueous solutions of various biologically active substances are aimed at the determination of hydration of these substances and of the contribution of the ionic and non-ionic group of such a substance in total hydration.

Our results of measurements of hydration numbers of sugars [27], dextran [28], nucleosides [29], amino acids and oligopetides [30] as well as of 1 : 1 electrolytes [31] were presented previously. This paper presents the results of measurements of hydration numbers of nucleic acids and nucleotides. The preliminary data on this subject were reported previously [32]. In order to examine the role of phosphate groups in the hydration of nucleic acids and nucleotides, the measurements of hydration numbers of monobasic sodium and potassium phosphates were also performed.

2. Experimental

The hydration numbers were determined from the measuerments of ultrasonic velocity in the aqueous ethanolic solutions. The method was described in details elsewhere [27, 33]. Sodium and potassium salts of nucleic acids DNA and RNA, and mono- and disodium salts of nucleotides were used. Sodium and potassium salts of nucleic acids as well as monosodium salts of nucleotides were prepared by slow neutralization of the acids with a 0.1 *n* base solution, using a slight excess of base. DNA $(C_{39}\ H_{51}\ N_{15}\ O_{25}\ P_4)_n$, *RNA* and 5′-citidine monophosphoric acid (CMP) were from Fluka AG; (5′-adenylic acid) 5′-adenosine monophosphoric acid (AMP) from Centrum Badań Medycznych, Łódź, Poland; disodium derivatives of nucleotides $AMPNa_2$, $UMPNa_2$, $CMPNa_2$, $GMPNa_2$ and $IMPNa_2$ from Fluka AG; 2(3′) -citidine monophosphoric acid from which 2(3′) $CMPNa_2$ was obtained, from Loba, Austria; inorganic phosphates from POCh, Gliwice, Poland.

Hydration of solute H (expressed in grams of water per gram of the solute) was determined from the relation:

$$H = W_x d_0 / m, \tag{1}$$

where W_x is the volume, in cm³, of water bound to m grams of solute and d_0 is the density of water. W_x is determined from the position of maximum of ultrasound velocity in the water-ethanol-solute system using the relation of YASUNAGA [34, 35]:

$$A_0/W_0 = A_1/(W_1 - W_x), \tag{2}$$

where A_0 and W_0 are the volumes of alcohol and water, respectively, at the point of maximum of velocity in the water-ethanol system; A_1 and W_1 are the volumes of alcohol and water at the piont of maximum in the water-ethanol-solute system.

The hydration number n_t (in moles of H_2O per mole of solute) is determined by the formula:

$$n_t = HM/M_0, \tag{3}$$

where M_0 is the molecular weight of water and M is the molecular weight of the solute.

In the case of nucleic acids n_t was related to the mean molecular weight of nucleotides in DNA and RNA.

The results are presented in Table 1 and 2. For comparative purposes hydration numbers of nucleosides and ribose [29], are placed in Table 2. The values of n_t were measured at 25° C. Assuming that the temperature dependence of n_t is similar to that found for 1 : 1 electrolytes, sugars, dextran, carboxylic acids, amino acids and peptides [27]–[33] the hydration number was extrapolated to 0° C using the relation:

Table 1

	M_w	H [g/g]	$n_t\left[\frac{\text{mole}}{\text{mole}}\right]$	$n\left[\frac{\text{mole}}{\text{mole}}\right]$	Error n_t
2/3′-$CMPNa_2$	367	1.09	22.2	20.4	±1.0
5′-$CMPNa_2$	367	1.04	21.2	19.4	±1.0
5′-$AMPNa_2$	391	0.98	21.3	19.5	±1.0
5′-$UMPNa_2$	368	1.04	21.3	19.5	±1.5
5′-$IMPNa_2$	392	1.02	22.2	20.4	±1.5
5′-$GMPNa_2$	407	0.95	21.5	19.7	±1.5

Table 2

	M_w	H [g/g]	$n_t\left[\frac{\text{mole}}{\text{mole}}\right]$	$n\left[\frac{\text{mole}}{\text{mole}}\right]$	Error n_t
AMPNa	369	0.68	14.0	12.2	±1.0
CMPNa	345	0.73	14.0	12.2	±1.0
DNANa	335	0.77	14.3	12.5	±1.5
DNAK	351	0.67	13.1	11.3	±1.5
RNANa	343	0.73	13.9	12.1	±1.5
RNAK	359	0.62	12.6	10.8	±1.5
NaH_2PO_4	120	2.22	14.8	13.0	±1.5
KH_2PO_4	136	1.81	13.7	12.0	±1.5
D-ribose [29]			4.1	2.3	
uridine [29]			2.7	0.9	
cytidine [29]			2.4	0.6	

$$n = n_t - At - Bt^2, \tag{4}$$

where n is the number of water moles per mole of solute at 0° C, A and B are experimental coefficients equal, respectively, to 0.0383 and 0.0013 and t is the temperature in °C.

3. Discussion

The studies on hydration of sugars [27] revealed that the values of n for various sugars are nearly equal to the average numbers of equatorial groups in cyclic forms of these sugars. According to a model of "specific hydration of sugars" [36], the position and orientation of equatorial $-OH$ groups facilitate hydration interactions of these groups with the molecules of water. If only equatorial groups $-OH$ are hydrated, from the data reported previously [27, 29, 36] it follows that each of these groups is hydrated by one water molecule.

Hydration number of D-ribose $n = 2.3$ (Table 2) is approximatly equal to an average number of equatorial $-OH$ groups. Uridine and cytidine nucleosides have identical hydration numbers, $n = 1$. In nucleosides one equatorial $-OH$ group of the sugar is replaced by a basic pyrimidine group. Hydration number of deoxyuridine deficient in one equatorial hydroxyl group as compared to uridine is equal to zero. Consequently, it can be stated that specific interactions of basic parts of nucleosides with water do not exist; if they exist then in an aqueous ethanolic solution they must be much weaker than water-water or water-ethanol ones. Thus in hydration of nucleosides the main part is played by the sugar component of nucleosides; the basic component is not hydrated. Basic components of nucleosides are not hydrated above all because of their inability to fit into the water lattice since rings of pyrimidine bases are located perpendicularly to the ribofuranose ring which, according to the model of "specific hydration" is built into octahedral water lattice. This inability to fit into the water lattice may result in the process of autcassociation of basic components of nucleosides.

The foregoing conclusions are fully confimed by the obtained results. As shown in Table 1 the obtained values of hydration numbers of $UMPNa_2$, $AMPNa_2$, $CMPNa_2$, $GMPNa_2$ and $IMPNa_2$ are identical within the limits of experimental error ($n = 20$). Therefore hydration numbers of nucleotides are independent of the type of the basic group in the compound. Identical hydration numbers $n = 12$ of CMPNa and AMPNa lead to the same conclusion. Subsequently, comparison of hydration numbers of CMPNa and AMPNa to those of NaH_2PO_4 and KH_2PO_4 indicates that the monovalent cations and phosphate anions of these substances interact with water in a similar way. This also confirms the thesis that basic components of nucleotides are not hydrated. Comparison of hydration numbers of DNANa, DNAK, RNANa and RNAK to those of CMPNa and AMPNa as well as to those of inorganic phosphates suggests the same conclusion. Assuming that sugar components of nucleotides are hydrated in the same way and in the same number ($n = 1$) as in nucleosides it may be concluded that in disodium salts of nucleotides ca. 19 water molecules are bound to sodium ions and the phosphate group $-PO_4^{-2}$. Analogically the number of water molecules bound to the sodium ion and phosphate group $-PO_4H^-$ in CMPNa and AMPNa is equal to 11. Assuming that the hydration number of the sodium ion is equal to 5 [31], the calculated hydration numbers of $-PO_4H^-$ and $-PO_4^{-2}$ are 6 and 9, respectively, and that of the $H_2PO_4^-$ ion is 8 (assuming that the hydration number of Na^+ is the same and that of K^+ is 4 [31]). Similar calculations performed for DNANa and DNAK give the hydration number of the phosphate group equal to 7. For RNANa and RNAK, having taken into account water bound to sugar component, the hydration number of the $=PO_4^-$ group equal to 6 is obtained.

Analysing the obtained results and comparing them with those obtained by others it must be stated that the lack of information about the quality of samples, about the degree of denaturation of DNANa, RNANa, DNAK and RNAK samples obtained by neutralization of the acids with the bases as well as about the structures of DNA and RNA in a mixed ethanol-water solvent does not allow us to draw far reaching conclusions about the structure of water in the vicinity of these macromolecules. Nothwithstanding, by comparing the hydration numbers of alkali metal salts of nucleotides with those of alkali metal salts of phosphoric acid certain observations on interactions of these substances with water can be made:

1° Low values of hydration numbers of DNA and RNA alkali metal salts, similar to those of monobasic sodium and potassium phosphates, indicate that these numbers take into account the water which is bound to primary hydration centers ($=PO_4^-$, Me^+) [37].

2° Nearly indentical values of hydration numbers of DNANa, RNANa as well as of CMPNa and AMPNa suggest that the same concept of their building-in into water lattice should be assumed. According to this concept metal ions, phosphate groups and equatorial $-OH$ groups (if present) orient themselves towards the cages of the icelike structure of water, while pyrimidine bases rings located perpendicularly to ribofuranose rings undergo autoassociation.

As it results from our calculations, a phosphate group binds 6–7 water molecules. This result is in good agreement with the results obtained from experimental studies carried out with the use of other techniques as well as from theoretical calculations. The hydration number of the phosphate group of *DNA* determined by infrared spectroscopic analysis is 4–6 [5, 37, 38]. Wolf and Hanlon [39] found this number to be equal to 6. Quantum-mechanical calculations performed by Pullman et al. [6, 7, 10] give the number of water molecules in the first hydration layer of phosphate group as equal to 6. The same number for the first hydration layer was obtained by Clementi et al.

[12] from the calculations carried out by means of the Monte Carlo method. The latest NMR studies of DNA [40] show that 5 water molecules are bound to a phosphate group. The experimental data concerning the hydration of phosphate groups also come from the studies of phospholipides. KLOSE et al. [41, 42] established the hydration number of a phosphate group to be 5 by the ^{2}D NMR method applied for aqueous solutions of dipalmitylphosphatidylcholine. CEVC [43] determined hydration numbers of the $=PO_4H$ group and the $=PO_4^-$ ion as equal to 5 and 7–8, respectively, by studying a group of various phospholipids.

The presented measurement results of hydration numbers of alkali metal salts of nucleic acids are also in agreement with theoretical models given in [44, 45], which explain the role of alkali metal ions in hydration and structure stabilization of DNA. On the basis of the data concerning 1 : 1 electrolytes it was suggested that the hydration number of a sodium ion is equal to 5, not to 6, assuming that similarly to inorganic salt solutions the hydrated sodium ions dispose around the phosphate groups forming with them ionic pairs through one or more polarized water molecules. Mixed alcohol-water solvent of a dielectric constant about 60–70 is undoubtedly a factor facilitating such interactions. The value of the hydration number of a sodium ion lower by 1 results from the assumed principle [31] that a common water molecule binding cation to anion is ascribed to hydration layer of anion. The same values of the hydration number of a sodium ion (5–6) are assumed in [44, 45] and a hydrated sodium ion is thought to locate in a narrow fissure of DNA bihelix, forming a hydrogen bond with phosphate groups.

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Received on 26 November, 1984; revised version on 31 May, 1985.