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МОЛЕКУЛЯРНА БІОФІЗИКА

RELAXATION AND SPECTROSCOPIC MANIFESTATIONS OF HYDRATION OF POLYRIBOCYTIDYLIC ACID

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Hydration and structure state of polyribocytidylic acid (poly(rC)) have been studied in solutions and humid films. The role of water in forming different structures of poly(rC) was elucidated. In solutions, the hydration values of single-strand (pH 4.5), double-strand (pH 7.8) and disordered (pH 3.3) forms of poly(rC) were determined with a differential EHF-dielectrometer using the measurements of dielectric permittivity at wavelength 7.6 mm. In humid films, distribution of water molecules on the hydration sites for these structures has been found with IR spectroscopy. Hydration models of the single and double-strand structures of poly(rC) were developed using X-ray and these data.

KEY WORDS: polycytidylic acid, hydration, infrared spectra, millimeter region, structural transitions.

The use of dielectric measurements in the range of millimeter range (extremely high frequencies (EHF)) is important in a number of areas of biology and medicine. In particular, they allow one to study the state of water in biological solutions [1].

The methods of X-ray diffraction analysis, NMP, IR and CD spectroscopy, dielectrometry and others have established the presence of bound water around biomacromolecules [2]. Such solvent molecules attach to the hydration-active sites on the biopolymer surface through hydrogen bonding. In energy terms the water molecule may be considered «bound» if its energy of interaction with any hydration center exceeds the mean energy of water-water interaction in the liquid phase.

Numerous investigations demonstrated that without detailed knowledge of the features of the interaction of biomolecules with water there could be no complete understanding the properties and behavior of the macromolecules. Because the bound molecules have a lower mobility, the corresponding relaxation peak is observed near 100 MHz while the peak due to ordinary water locates around 20 GHz [3].

Unfortunately, the dispersion of dielectric permittivity of biomolecules is also observed near 100 MHz creating substantial difficulties in the interpretation of dielectric data. On the other hand, in the millimeter range the dispersion observed is only due to the relaxation of bulk water and hydration leads to a lowering of the dielectric permittivity of solution as compared with that of solvent [1]. This decrement can be used to study the interaction of biomolecule with water.

The present work is devoted to the investigation of the hydration environment existing around poly(rC) (polyribocytidylic acid). This polynucleotide is known to form different structures in aqueous solutions as a function of the pH, temperature and ion content [4]. At room temperature and neutral pH, poly(rC) is an ordered single-strand helix. In the range $3.7<pH<5.5$ it forms a helical double-strand complex in which a proton is added to the ring nitrogen for each pair of bases (Fig.1). Below pH 3.5, through the addition of a second proton per base pair, a dissociation into two disordered chains with protonated cytosines occurs. It is believed that the hydrogen bond between the protonated and unprotonated rings mainly stabilizes the hemiprotonated complex.

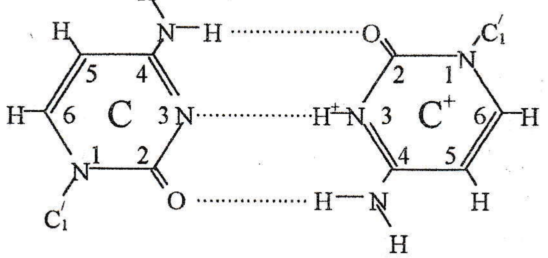

Figure 1. Hydrogen bonding between bases in the hemiprotonated complex.

The aim of the work is to elucidate the role of water environment in the formation of the different structures of poly(rC).

MATERIALS AND METHODS

We used samples of poly(rC), K^+ salt, obtained from «Serva» (Germany) and «Reanal» (Hungary). The content of the K^+ ions in relation to the dry mass of the biomolecule was 6.7%(«Serva») and 8.5%(«Reanal»). Solutions in the range $3<pH<5.5$ were prepared by the addition of HCl. For microwave measurements, 1% solution was used. For infrared studies, thin uniform films of poly(rC) were prepared and deuterated as described previously [5]. The wet films in the relative humidity (r.h.) interval from 0 to 96% were derived in the special cuvette. The degree of protonation was constant up to r.h.=86%.

Measurements in millimeter range were carried out by the differential dielectrometric method developed by us [6]. The decrement of the complex dielectric permittivity $\Delta\varepsilon^x = \varepsilon^*_{solvent} - \varepsilon^*_{solution} = \Delta\varepsilon' - i\Delta\varepsilon''$ was determined. The knowledge of the complex permittivity and conductivity σ measured at f=10kHz made it possible to calculate the effective dielectric constant of solution ε_s which is characteristic parameter of the Debye theory of fluids

$$\varepsilon_s = \varepsilon' + \frac{\left(\varepsilon'' - \sigma/f\right)^2}{\varepsilon' - \varepsilon_\infty}, \qquad (1)$$

where ε_∞ is the high-frequency limit of dielectric permittivity. The validity of the Debye equation to the description of the relaxation of pure water was shown in a number of works [3].

The IR spectra were recorded with the UR-20 two-beam spectrometer, NaCl prism, spectral slit width 6 cm^{-1} at 1700 cm^{-1}.

RESULTS AND DISCUSSION

Table 1 presents the results of dielectric measurements. The initial experimental values are the differences between the attenuation $\Delta\alpha$ and phase shifts $\Delta\beta$ of the solvent and solution. From these findings the analogous differences $\Delta\varepsilon'$, $\Delta\varepsilon''$ and $\Delta\varepsilon_s$ have been determined (for water ε'=18 and ε''=28.5 at 23°C). Experimental errors for $\Delta\varepsilon'$ and $\Delta\varepsilon''$ are within 0.02 and 0.03, respectively.

The degree of hydration ω (g water per g solute) or n (the number of bound water molecules per nucleotide) may be found from the data obtained. Its value is proportional (in the first approximation) to the dielectric decrement and can be derived as

$$\omega = \Delta\varepsilon_s / p \cdot c \cdot \left(\varepsilon_s - \varepsilon_\infty\right) - v\,, \qquad (2)$$

where c is the concentration of poly(rC), v is the specific volume of poly(rC), p is coefficient depending upon the form of the polymers. According to the earlier paper [7], its meaning varies from 1.5 (sphere) to 1.66 (cylinder). Recently, it became possible to obtain the degree of DNA hydration (B form) using the dielectric measurements in the range of dispersion of bound water [3]. This value (n =19) is in agreement with our estimation of DNA hydration if the value of p is assumed to be 1.65. All the values of n in the Table have been calculated using this value.

Table 1. Results of the dielectric measurements (T=23°C) of poly(rC)

Parameters measured	*pH 8* *c=1.18%*	*pH 4.5* *c=0.97%*	*pH 3.3* *c=1.3%*
$\Delta\alpha$ (nep/mm)	0.0161	0.0114	0.0151
$\Delta\beta$ (rad/mm)	0.0168	0.0164	0.0120
$\Delta\varepsilon'$	0.07	0.10	0.04
$\Delta\varepsilon''$	0.07	0.09	0.25
r/f	0.12	0.17	0.32
$\Delta\varepsilon_s$	1.34	1.26	2.16
$n(\pm 1)$	7-8	8-9	11

The millimeter measurements demonstrate that at pH 8 the amount of water molecules which got into strong interaction with single-stranded poly(rC) is equal to 7-8.

IR spectroscopy in wet films make it possible to study the stages of the formation of different structures of biopolymers and their hydration shells. Analysis of the changes in IR spectra occurring with wetting allows one to find the atomic groups which are hydration centers and to determine the order and degree of their fitting by water molecules.

To obtain such information on poly(rC) we derived IR spectra of the films for different humidities in the region of absorption of the intra- and the extraring vibrations of bases (1500-1750 cm^{-1}) and in the region of absorption of ribose and phosphates PO_2^- (900-1350 cm^{-1}). The dependences of the frequencies ν and intensities R on n were found from these spectra.

At the initial stage of sorption (0<r.h.<44%) the results of IR studies in the above regions point to the bonding of water molecules with such centers as atoms of the N_1, N_3 and, possibly, the groups C_2=O and NH_2 (base), O_4, and OH (ribose), PO_2^- (phosphate).

With further change in n in the interval 44%<r.h.<86% (to $n \cong 10$), the IR hypochromism and high-frequency shifts of the bands of the intraring vibrations and also the appearance of the band at 1292 cm^{-1} appear to be due to the ordering of cytosine rings and to the transition of poly(rC) to the fully ordered single-strand structure.

Thus, EHF (solution) and IR (wet films) results at pH 8 are close to each other and show that 8-10 bound water molecules are necessary to form the ordered structure of poly(rC).

From the dielectric data presented in Table 1, it follows that at pH 4.5 the hydration of poly(rC) is 8-9 molecules of water per nucleotide (double-stranded helical structure).

The dependences of ν and R on n (pH 4.6, $0<n<2\text{-}3$) for the absorption bands associated with the intra- and the extraring vibrations of cytosine may also be explained by hydration. The hydration centers are the same as those at neutral pH region and take part in the hydrogen bond between the cytosines of two opposite poly(rC) chains.

The rise of the intensity of the band at 970 cm^{-1} as well as the appearance of the bands at 1059 cm^{-1} and 1293 cm^{-1} ($0<n<5$) of ribose correspond to the formation of bonds between water molecules and the atoms $O_{4'}$ and OH. The water bridges connecting these atoms and phosphates, cytosines and atoms $O_{2'}$ and $O_{5'}$ of neighbouring strands are formed. This process stabilizes the double-helical complex.

Further wetting ($2<n<7$) leads to the changes in intensity and position of some bands associated with vibrations of bases and ribose. These effects indicate the formation of the double-helical conformation. Final stabilization of the complex takes place with increase in the water content to n=9.

This value fully agrees with the EHF findings. Thus, this amount may be considered to be necessary for the stabilization of double-helix poly(rC) both in solution and in films.

Dielectric measurements show that the poly(rC) hydration at pH 3.3 is 11 water molecules per nucleotide. Since in this case the double-stranded complex dissociates with elimination of base pairing, water bridges and ordered helical conformation, it might be anticipated that the hydration at pH 3.3 might not exceed the hydration at pH 8. The greater value which is observed is apparently due to the protonation of N_3 (65% at pH 3.3). It is known that the hydration number for proton is 10 [8], so that the poly(rC) chain, except N_3, binds 5 water molecules. This value is very close to IR data (n=6). However, the latter value may also be explained by aggregation of poly(rC) in acid region (pH<3).

CONCLUSIONS

Analysis of EHF and IR studies demonstrates that hydration of the poly(rC) helical forms is about 10 water molecules per nucleotide. The hydration sites of poly(rC) are atomic groups C_2=O, NH_2, $O_{4'}$ N_1, N_3 and PO_2^-. It is shown that the binding of water molecules together these groups plays a main role for the formation of the helical structures of poly(rC).

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РЕЛАКСАЦІЙНІ ТА СПЕКТРОСКОПІЧНІ ПРОЯВИ ГІДРАТАЦІЇ ПОЛІРИБОЦИТИДИЛОВОЇ КИСЛОТИ

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В роботі досліджено гідратацію та структурне становище полірибоцитидилової кислоти (полі-рЦ) в розчинах та вологих плівках. Визначено роль води у формуванні різних структур полі-рЦ. За допомогою диференційного НВЧ-діелектрометра виміряно діелектричну проникність розчинів полі-рЦ на довжині хвилі 7,6 мм. Знайдено гідратаційні числа односпіральної (pH=4,5), двохспіральної (pH=7,8) та неупорядкованої (pH=3,3) форм полі-рЦ. Центри гідратації цих структур винайдено з інфрачервоних спектрів вологих плівок. За цими та рентгенівськими даними розвинуто гідратаційну модель одно- та двохспіральної структури полі-рЦ.

КЛЮЧОВІ СЛОВА: полірибоцитидилова кислота, гідратація, інфрачервоні спектри, міліметровий діапазон, структурні переходи.