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THE EFFECT OF HYDRATION AND INTERACTION WITH D-MANNITOL ON THE SPECTRAL CHARACTERISTICS OF PROTONATED AND DEPROTONATED MONORIBONUCLEOTIDES AND THEIR CONSTITUENTS

Background. Recent studies have revealed clear difference between the electronic structure of organic molecules in water, in dry state, and within a cell. Consequently, the properties of active pharmaceutical ingredients *in vivo* and in water – where most structural studies are conducted – differ significantly. However, there are no spectral studies examining the effect of hydration on nucleotides and their constituents. To investigate this effect, we measured and compared the spectra of the main nucleotide bases of RNA, their bases, nucleosides, protonated and deprotonated forms of monoribonucleotides and their mixtures with D-mannitol in the dry state and in their aqueous solutions. **Methods.** Measurements of 3D spectra in dry state and in water were performed using Horiba FluoroMax 4P+ instruments (HORIBA Scientific Jobin Yvon, USA). The experiments were conducted at 20 °C, and the concentration of a sample in deionised water was 1 mg/ml. **Results.** Hydration led to a shift in the fluorescence maxima, suggesting changes in the electronic energy levels due to interaction with water molecules. For example, hydration of adenine resulted in a shift of the fluorescence band by 115 nm. Hydration of nucleotides with purine bases resulted in a shift of the fluorescence bands and an 8-fold increase in fluorescence intensity for AMP and a 15-fold increase for GMP. Their disodium salts showed a shift in the fluorescence maxima, but no increase in intensity. Furthermore, no signs of interaction between the deprotonated forms of monoribonucleotides and D-mannitol were observed, either in the dry state or in water. Among all bases and nucleosides tested, the interaction with D-mannitol was most pronounced in the spectrum of the aqueous cytosine solution: the addition of D-mannitol caused a shift in the emission band of 62 nm. Among the nucleotides, the greatest effect of D-mannitol was observed in protonated AMP and GMP (a shift of 77 nm in AMP and the appearance of a new peak in GMP). **Conclusions.** We have investigated for the first time the effect of water and D-mannitol on the fluorescent properties of protonated and deprotonated monoribonucleotides and their components. Changes in the spectra indicate structural changes in the molecules under the influence of the hydration shell. The effect of hydration was greatest for protonated AMP and GMP, which have purine bases. The effect of D-mannitol was greatest on aqueous solutions of cytosine and protonated AMP and GMP.

Keywords: nucleotides, D-mannitol, hydration.

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