STABILITY OF PROTONATED FORMS OF MONONUCLEOTIDES IN ALKALINE ENVIRONMENTS

M.A. DOTSENKO[1]*, R.O. NIKOLAIEV[1], Z.YU. TKACHUK[1]
[1]Institute of Molecular Biology and Genetics of NASU, 150, Ac. Zabolotnogo St., Kyiv, Ukraine, 03143
E-mail: mychailodotsenko@gmail.com

Introduction

It is believed that in a weakly alkaline intracellular environment, mononucleotides can only exist in a salt (deprotonated) form, while protonated forms quickly transition to a salt state. Consequently, salt forms of mononucleotides are regarded as having significant potential for research and medical applications. However, studies conducted in our laboratory have shown clear antiviral, antitumour and anti-inflammatory activity of protonated forms of 5'-mononucleotides, which their salt (deprotonated) forms did not have[1]. We suggested that this activity could be explained by a significantly lower than previously thought rate of transition of 5'-mononucleotides to a deprotonated state in alkaline environments.

Our previous studies were the first to demonstrate differences in the fluorescence of aqueous solutions of protonated and deprotonated forms of 5'-ribonucleotides[2]. We used these differences to identify the form of nucleotides dissolved in weakly alkaline buffers.

Aim

The aim of the study was to identify differences in the spectral characteristics of protonated and deprotonated forms of 5'-mononucleotides, as well as to assess the stability of protonated forms of 5'-mononucleotides in weakly alkaline environments.

Material and Methods

We used Horiba Fluoro Max4+ instruments to measure fluorescence spectra. We studied solutions of 5'-ribonucleotides in protonated form (5'-AMP, 5'-GMP, 5'-CMP, 5'-UMP) and their disodium salts in 1x PBS and 0.05 M Tris (pH 7.4–7. 5) at concentrations of 2.9 and 14.5 mM at a temperature of 30 °C. The spectra were measured for 80 minutes after dissolution. The fluorescence spectra of the protonated forms were compared with the corresponding spectra of disodium salts to assess their stability in weakly alkaline environments.

Results and Discussion

Measurements showed clear differences in the fluorescence of protonated and deprotonated (salt) forms of 5`-mononucleotides dissolved in alkaline buffers.

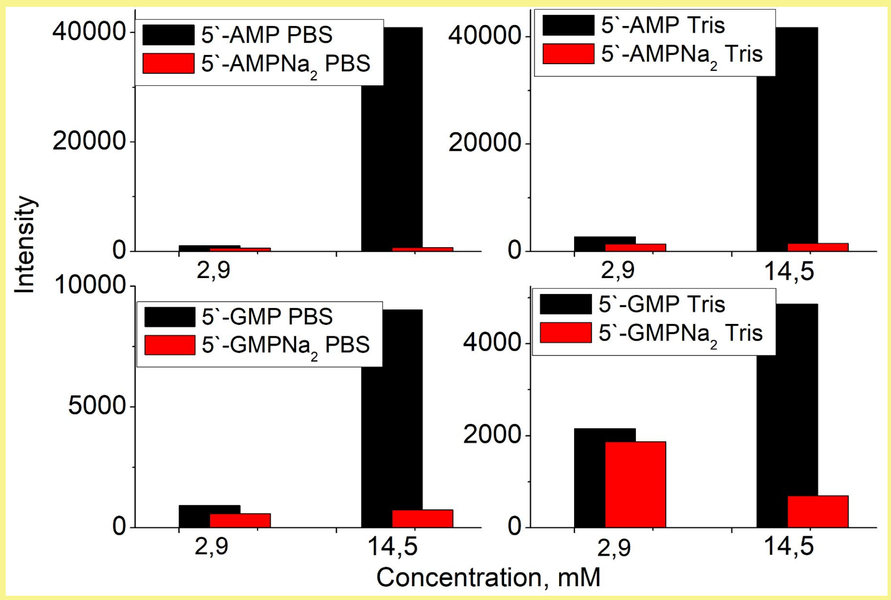

Figure 1 *Comparison of the fluorescence intensity of protonated and salt forms of 5'-adenosine monophosphate and 5'-guanosine monophosphate dissolved in PBS and Tris alkaline buffers at different concentrations*

The most significant differences were related to fluorescence intensity, which was much higher in protonated forms (except for 5'-CMP). The dependence of fluorescence on concentration also differed, as shown in Figure 1. For example, when the concentration of the protonated form of 5'-adenosine monophosphate was changed from 2.9 to 14.5 mM in PBS buffer, its fluorescence intensity increased 30-fold. At the same time, the fluorescence intensity of 5'-adenosine monophosphate salt remained almost unchanged. In addition, the fluorescence characteristics of nucleotides with purine and pyrimidine bases differed. 5'-AMP and 5'-GMP showed greater differences in fluorescence between protonated and salt forms than 5'-CMP and 5'-UMP, which have pyrimidine bases.

Also, 5'-nucleotides of protonated forms showed high stability in alkaline buffers. During the entire measurement time (more than 80 min), no transition of the spectra of protonated forms to the spectra of salts was observed. The spectra of protonated and salt forms of 5'-AMP dissolved in PBS and Tris buffers are shown in Figure 2:

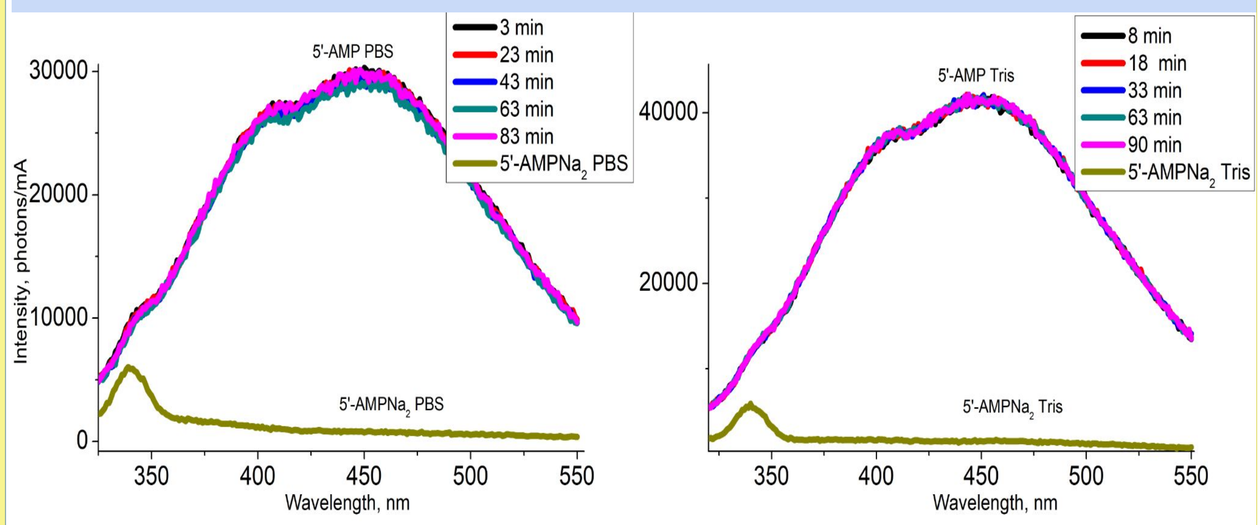

Figure 2 *Fluorescence spectra of protonated and deprotonated forms of 5'-adenosine monophosphate depending on time after dissolution in alkaline PBS and Tris buffers*

Conclusions

Clear differences were found in the fluorescence spectra of protonated and salt forms of 5'-mononucleotides. These differences were evident throughout the entire measurement period. Thus, we have shown that protonated forms of 5'-mononucleotides can remain stable for 80 minutes in alkaline buffers. This makes protonated forms of 5'-mononucleotides promising for further drug development.

References

[1] Tkachuk Z. Compound, composition and method for the treatment of inflammatory and inflammatory-related disorders: пат. 6,737,271 B1/Biocell Laboratories Inc. - США - опубл. 18.05.2004

[2]Dotsenko, M.A.; Nikolaiev, R.O.; Tkachuk, Z.Yu. Spectral analysis of protonated and deprotonated form of ribonucleotides and their components at room temperature. *Nucleosides, Nucleotides & Nucleic Acids* **2025** pp. 1–16. DOI 10.1080/15257770.2025.2521555