Insights into acid-base properties of thiamine and its phosphate derivatives by spectroscopic and *ab initio* studies

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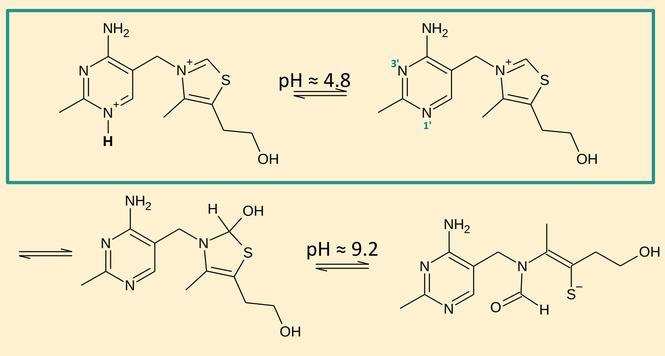
Introduction



Scheme. 1. pH dependent forms of thiamine [1]. Proton dissociation (reaction in the frame) of thiamine and its phosphate derivatives is the objective of this study.

Thiamine (Th, B1) is a water-soluble vitamin essential to metabolic processes occurring in living organisms. In nature, there exist phosphorylated derivatives of the vitamin B1 containing one, two or three phosphate groups instead of the hydroxyl group (Thp, Thpp and Thppp, respectively). The biologically active form is thiamine diphosphate, serving as a cofactor for a group of enzymes involved in carbohydrate metabolism. In acidic conditions, thiamine has two positive charges – one on the nitrogen of pyrimidine ring and one on thiazole ring. The pyrimidine moiety undergoes deprotonation upon increasing pH to become a singly charged cation at physiological conditions. In alkaline solution, thiazole ring is hydrolyzed, which leads to the ring opening and formation of a negatively charged thiol [2] (Scheme 1.). Both the ionic equilibria and the tautomeric states coupled with the protonation shift have the biological importance [3].

The aim of this study is to evaluate the effect of the presence of phosphate groups in thiamine on its pK_a and comparison of pK_a between the ground state and the excited state. For this purpose spectroscopic methods have been employed. In order to better understand these relations, the support of *ab initio* computations can be useful.

Structures

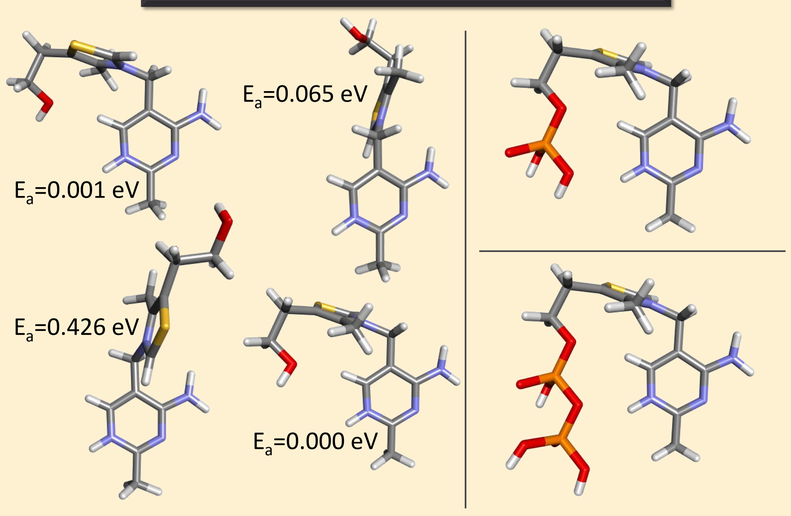


Fig. 1. Examples of N(1') protonated thiamine conformers in a ground state optimized by using MP2/cc-pVDZ *ab initio* calculations with energies relative to the most stable conformer. Phosphate groups were added to the lowest energy thiamine conformer to be further optimized.

Measurements

UV absorption spectra

The absorption spectra measurements were carried on Varian Cary 50 UV-Vis spectrophotometer in a quartz cell (light path l=10 mm), after being thermostatted (± 0.2 °C) to 20 °C. Samples were measured in Britton-Robinson buffers of ionic strength I=0.1 [4] (maintained with NaCl) in the pH range from 2.83 to 7.76 in 2 series. Solute concentrations were equal to 60 µM. pH dependence of absorption spectra was expressed by absorbance at 246 nm (λ_{max} of the N(1') protonated form) and corrected for changes in concentration by dividing by absorbance at isosbestic point (236 nm). Acid-base equilibria of thiamine and its phosphate derivatives were then determined by nonlinear regression of the plotted points (Fig. 3.) based on equation:

$$y = B + \frac{A-B}{10^{x-pKa+1}}$$

Fluorescence spectra

The fluorescence spectra were measured on Fluorolog 3.11 in a 4x10 mm cell with magnetic stirring thermostatted (± 0.2 °C) to 20 °C. Excitation wavelength was 250 nm. Buffers and concentrations were the same as those used for absorption measurements, samples were measured in 2 series. pH dependence of fluorescence was expressed as integrated emission spectra over λ^{-1} in the range of 310-380 nm and corrected for absorption at excitation wavelength (250 nm) and inner filter effect. Pk_a^* of the measured compounds in an excited state were determined accordingly to the pK_a in a ground state, by fitting the equation above to the points in Fig. 5.

References

[1] Pérez-Caballero G., *et al.* (2011). Potentiometric Study of acid-base properties of thiamine hydrochloride and thiamine mononitrate in aqueous medium. *J. Mex. Chem. Soc,* 55, 126-131.; **[2]** Edwards K. A., *et al*. (2017). Thiamine assays – advances, challenges, and caveats. *ChemistryOpen*, 6, 178-191.; **[3]** Balakrishnan A., *et al*. (2012). Solid-state nuclear magnetic resonance studies delineate the role of the protein in activation of both aromatic rings of thiamin. *J. Am. Chem. Soc.*, 134, 665-672.; **[4]** Mongay C., Cerdà V. (1974). *A Britton-Robinson buffer of known ionic strength*. Annali di Chimica, 64, 409-412.; **[5]** Wieczorek Z., Stepinski J., Jankowska M., Lönnberg H. (1995). Fluorescence and absorption spectroscopic properties of RNA 5'-cap analogues derived from 7-methyl-, N^2,7-dimethyl- and N^2,N^2,7-trimethyl-guanosines. *J Photochem. Photobiol. B.*, 28, 57-63.; **[6]** Driscoll E. W., Hunt J. R., Dawlaty J. M. (2016). Photobasicity in quinolines: origin and tunability *via* the substituents' Hammett parameters. *J. Phys. Chem. Lett.*, 7, 2093-2099.

Ground state

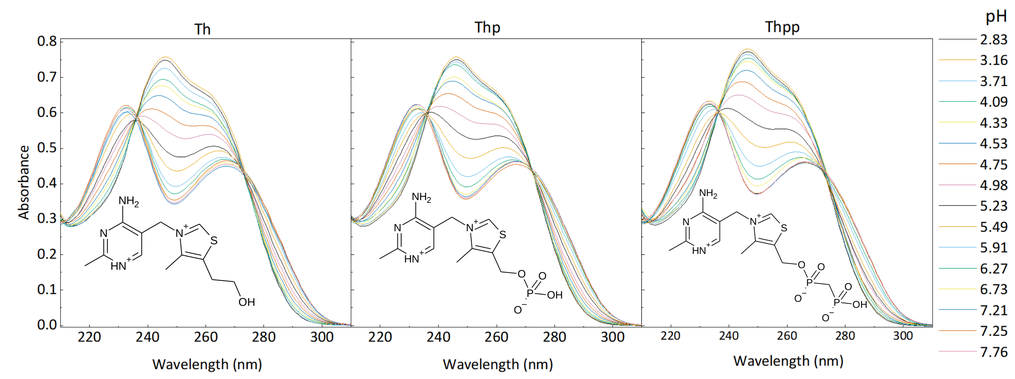


Fig. 2. pH dependence of the absorption spectrum of thiamine (Th), thiamine monophosphate (Thp) and thiamine diphosphate (Thpp).

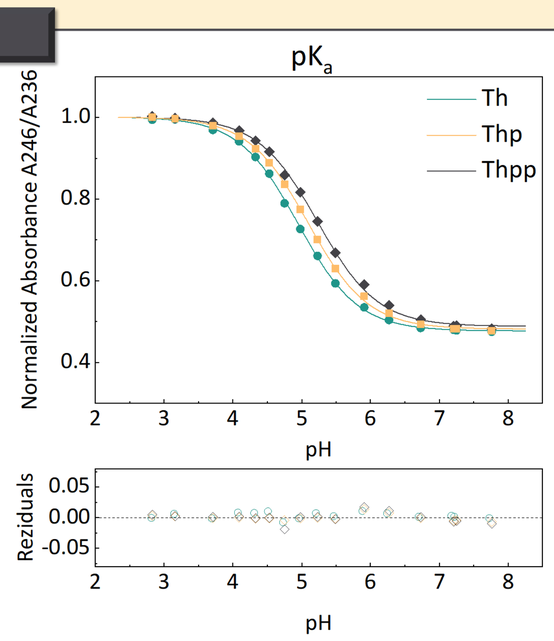


Fig. 3. pH dependence of absorbance at 246 nm divided by absorbance at 236 nm (isosbestic point) of thiamine (Th), thiamine monophosphate (Thp) and thiamine diphosphate (Thpp). The points on the graph represent the averaged value of 2 measurements for each pH.

Excited state

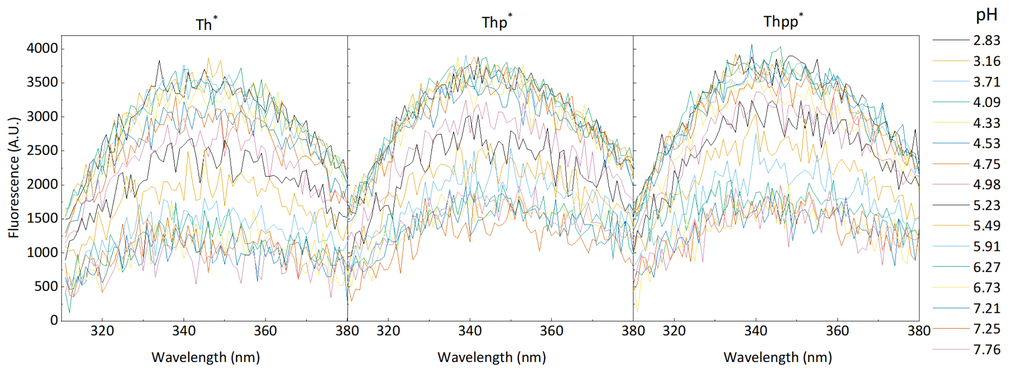


Fig. 4. pH dependence of the fluorescence spectrum of thiamine (Th), thiamine monophosphate (Thp) and thiamine diphosphate (Thpp), corrected for inner filter effect and absorption at excitation wavelength.

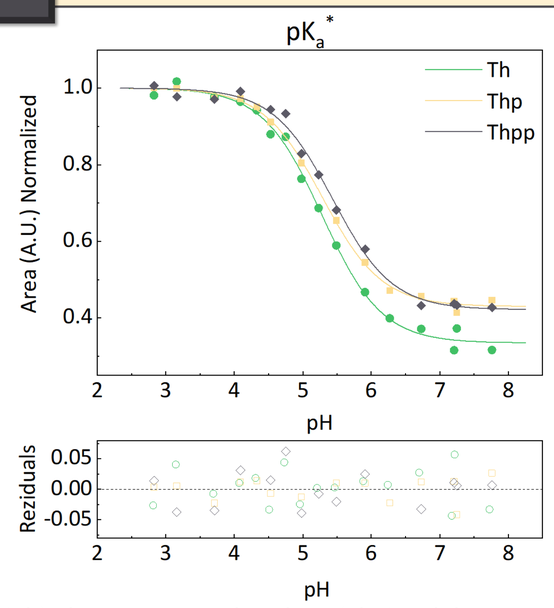


Fig. 5. pH dependence of the emission spectrum integrated over λ^{-1} of thiamine (Th), thiamine monophosphate (Thp) and thiamine diphosphate (Thpp) after corrections. The points on the graph represent the averaged value of 2 measurements for each pH.

Summarized experimental data

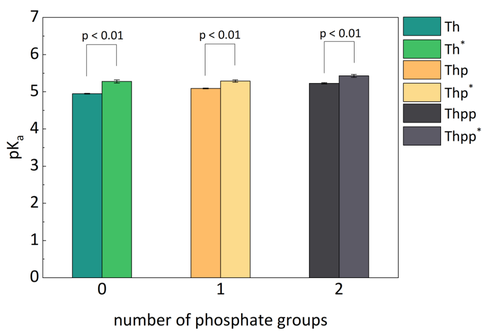


Fig. 6. pK_a of thiamine (Th), thiamine monophosphate (Thp) and thiamine diphosphate (Thpp) grouped by the number of phosphates.

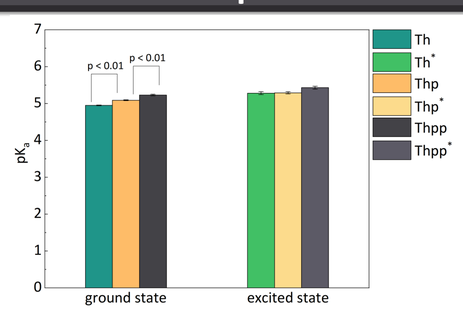


Fig. 7. pK_a of thiamine (Th), thiamine monophosphate (Thp) and thiamine diphosphate (Thpp) grouped by the ground state and the excited state.

Compound	pK_a	pK_a^*
Th	4.95±0.01	5.28±0.04
Thp	5.09±0.01	5.29±0.03
Thpp	5.23±0.02	5.43±0.04

Table 1. pK_a of thiamine (Th), thiamine monophosphate (Thp) and thiamine diphosphate (Thpp) in the ground state and the excited state.

Ab initio

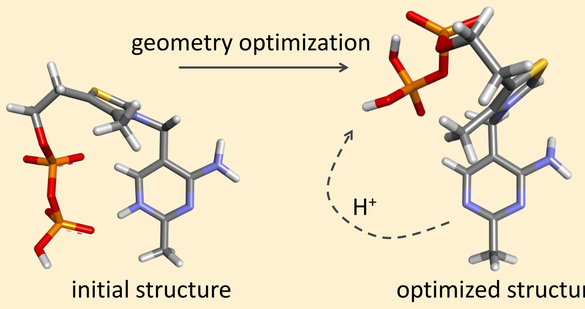


Fig. 8. Geometry optimization of N(1') protonated thiamine with partially protonated diphosphate moiety in its ground state (S_0, MP2/cc-pVDZ) resulting in proton transfer from N(1') nitrogen to the negatively charged oxygen on a phosphate.

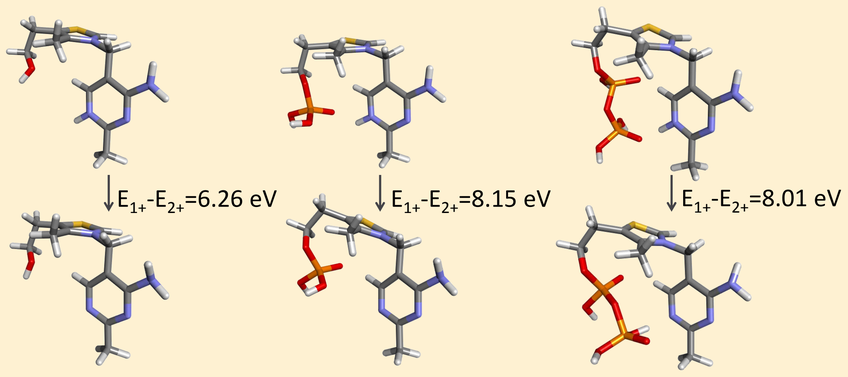


Fig. 9. Energy difference between N(1') unprotonated and protonated form of thiamine, thiamine monophosphate and thiamine diphosphate. The energies in a ground state were obtained from MP2/cc-pVDZ calculations.

Conclusions

- The presence of phosphate moiety on the imidazole side chain increases the basicity of N(1') nitrogen on the pyrimidine ring of thiamine phosphate derivatives. The trend is less pronounced in the excited state. The same effect of phosphates present in a compound has been observed in mRNA 5' cap analogues [5].
- pK_a of the excited state is increased compared to the ground state indicating photobasicity of thiamine. This phenomenon has not been recognized in cap analogues, however it is present in quinolines [6].
- Theoretical part of the experiment needs further investigation. Nevertheless, first computational results indicate that the presence of the partially protonated phosphate groups in thiamine leads to proton transfer from N(1') nitrogen to the negatively charged oxygen in vacuum.

Acknowledgements

Calculations were performed at the PL-Grid Infrastructure.