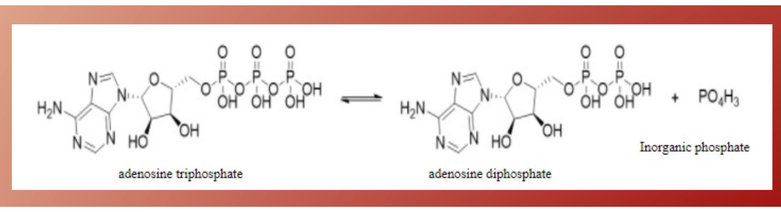
Qualitative Measurements of Stability of ATP at Varying pH

Tyler Deutsch, Mary Ruth Shifflett, and Dr. Barnabas Otoo

Department of Chemistry, Bridgewater College, 402 East College Street, Bridgewater, VA 22812-1599, USA

Introduction

Adenosine triphosphate (ATP) is the most important energy-carrying molecule. The hydrolysis of the phosphoanhydride bond in ATP releases energy to fuel the vital bioprocesses.



ATP is produced during cellular respiration as follows: the digestion of food molecules into intermediate molecules (such as pyruvate and acetyl-CoA), allow for electron carriers, NAD+ and FAD, to temporarily store energy. The electron carriers donate electrons to the electron transport chain within the inner mitochondrial membrane. As these electrons are passed through embedded proteins with increasing redox potentials, hydrogen ions (H+) are pumped against their concentration gradient into the intermembrane space. The proton motive force drives ATP synthase to synthesize ATP molecules.[1]

ATP is known to be relatively stable in basic conditions.[2] The pKa's of its three acidic protons were computed to be 6.6, 3.0 and less than 0.[3] This project seeks to provide a qualitative measurement of the stability of ATP at various pH.

Experimental

ATP was purchased from Sigma-Aldrich and refrigerated at –80 °C. UV measurements were taken on a Varian Cary 60 UV-Vis Spectrophotometer.

Calibration

1.378 g ATP and DI water were used to prepare 250 mL 10 mM ATP solutions. 50 mL of 5 mM, 2mM, 1mM, 0.5 mM, and 0.1 mM ATP solutions were prepared. The solutions ranging from 10.0 mM to 0.5 mM were then used to determine the sensitivity of the instrument. No sensitivity was observed for the instrument at concentrations less than 1mM.

Initial Test of ATP's Protonated Stability

10.0 mM solution was divided into seven 50 mL volumetric flasks. ATP was combined with one of the following, 1M HCl, 1M NaOH, or left untouched. Adjusted ATP solutions included pH conditions of 2.23, 4.02, 5.10, 7.05, 8.98, 10.2, and 11.78. Each condition was then diluted to 1.0 mM of ATP. Absorbance* spectrum of each pH solution was measured 3 times in one trial.

Extended Test of ATP's Protonated Stability

1.653 g ATP and DI water were used to prepare 300 mL 10 mM ATP solution. Six 100 mL beakers of 5 mM ATP solution was prepared with 25 mL 10 mM ATP solution and 25 mL DI water. ATP then experienced either an addition of 1.0 M HCl, addition of 2.0 M NaOH, or was left untouched. Adjusted ATP solutions included pH conditions of 2.04, 3.40, 5.95, 8.10, 10.82, and 11.77. Each pH solution was roughly divided into a 30 mL sample stored at room temperature at 21.3˚C and a 20 mL sample stored in the fridge at 9˚C. 3 separate trials of absorbance* spectrum for room temperature pH was collected at time zero. 1 trial of absorbance* spectrum for solutions stored at room temperature and those stored in the refrigerator was collected again after 24 hours and then after a week.

*Absorbances were a collect of lowest to highest pH absorbance measurement with a UV-Vis spectroscopy range of 200nm - 350nm.

Figures

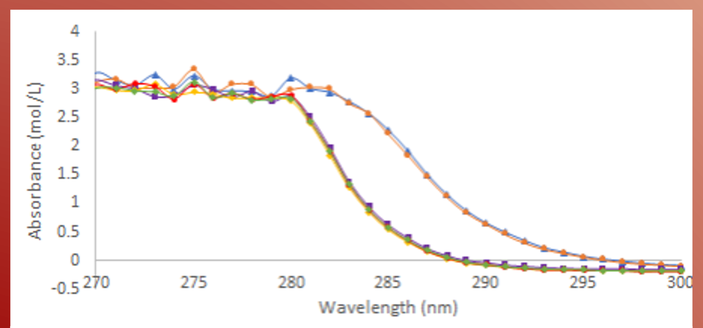
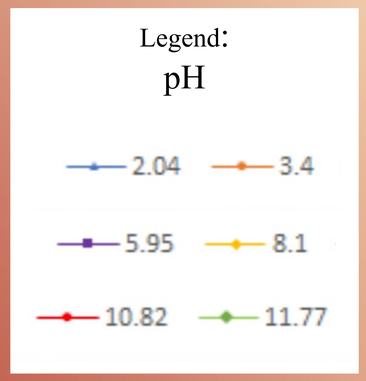


Figure 1. Initial ATP Absorbance at 21.3°C



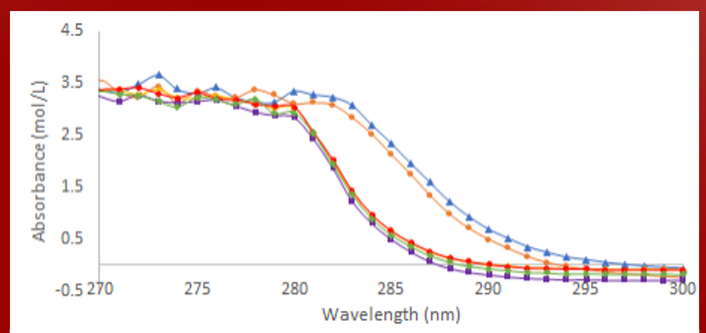


Figure 2. ATP Absorbance after 24 Hours Stored at 21.3°C

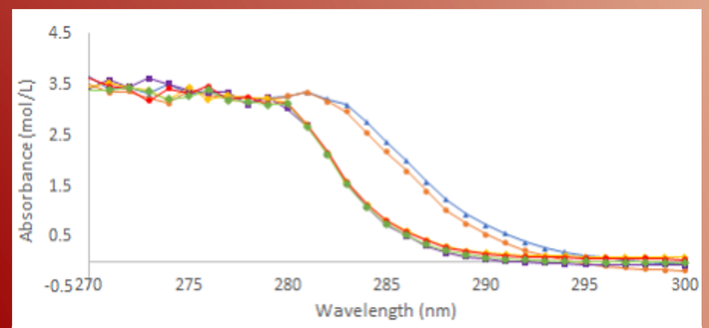


Figure 3. ATP Absorbance after 24 Hours Stored at 9.0 °C

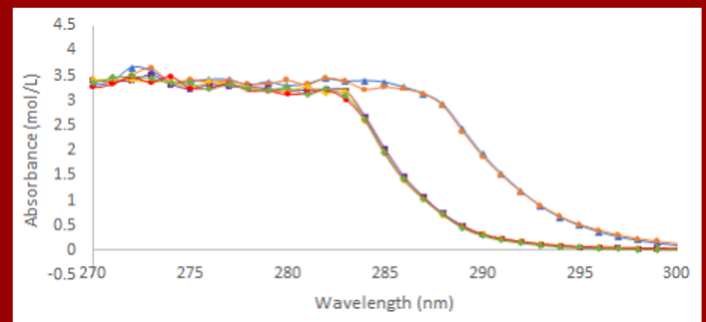


Figure 4. ATP Absorbance after 1 Week Stored at 21.3°C

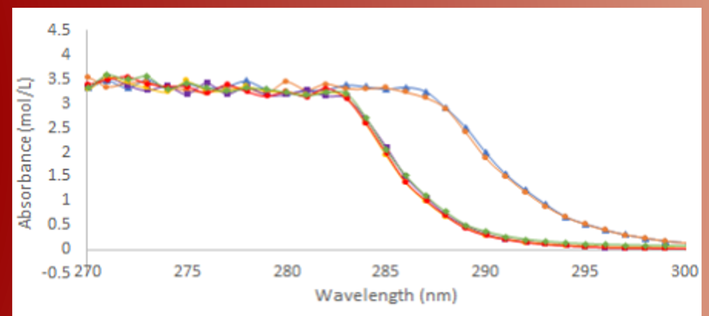


Figure 5. ATP Absorbance after 1 Week Stored at 9.0 °C

Results and Discussion

ATP solutions in acidic conditions (pH 2.04 and pH 3.40) deviated in absorbance spectrum at 260-280 nm from ATP in basics conditions (pH ≥ 5.95) in all cases. The deviation in absorbance spectrum confirms decomposition of ATP under acidic conditions specifically, at pH less than 5. The absorption pattern at pH higher than 5 remain constant in all cases. This indicates no significant decomposition in basic conditions.

Conclusion

Aqueous solutions of ATP are highly unstable under acidic conditions within seconds of preparation. ATP aqueous solutions are however very stable in higher pH conditions for long periods of time.

Acknowledgements

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References:

(1) Baron, S. (2021) *Lecture on Cellular Respiration*. Personal Collection of S. Barron, Bridgewater College, Bridgewater VA.

(2) Khalifat, N.; Puff, N.; Bonneau, S.; Fournier, J.; Angelova, M.; et al. Membrane Deformation under Local pH Gradient: Mimicking Mitochondrial Cristae Dynamics. *Biophysical Journal* 2008, 95, 4924-4933. https://doi.org/10.1529/biophysj.108.136077

(3) McElroy, W.D.; Glass, B. Phosphorus Metabolism. *John Hopkins University Press* 1951, 1.