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LABORATORY EXPERIMENT

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Adenine Synthesis in a Model Prebiotic Reaction: Connecting Origin of Life Chemistry with Biology

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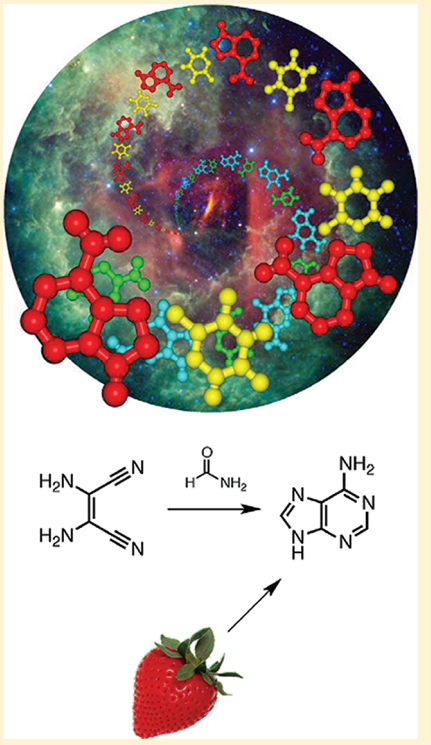
Supporting Information

ABSTRACT: Many high school laboratory experiments demonstrate concepts related to biological evolution, but few exist that allow students to investigate life's chemical origins. This series of laboratory experiments has been developed to allow students to explore and appreciate the deep connection that exists between prebiotic chemistry, chemical evolution, and contemporary biological systems. In the first experiment of the series, students synthesize adenine, one of the purine nucleobases of DNA and RNA, from plausibly prebiotic precursor molecules. Students compare their product to authentic standards using thin-layer chromatography. The second and third experiments of the series allow students to extract DNA from a familiar organism, the strawberry, and hydrolyze it, releasing adenine, which they can then compare to the previously chemically-synthesized adenine. A fourth, optional experiment is included where the technique of thin-layer chromatography is introduced and chromatographic skills are developed for use in the other three experiments that comprise this series. Concepts relating to organic and analytical chemistry, as well as biochemistry and DNA structure, are incorporated throughout, allowing this series of laboratory experiments to be easily inserted into existing laboratory courses and to reinforce concepts already included in any high school chemistry or biology curriculum.

KEYWORDS: First-Year Undergraduate/General, High School/Introductory ChemistryAnalytical Chemistry, Biochemistry, Organic Chemistry, Hands-On Learning/Manipulatives, Bioanalytical Chemistry, Bioorganic Chemistry, Nucleic Acids/DNA/RNA, Thin Layer Chromatography



The synthesis of biological molecules from simple, plausibly prebiotic precursors has been documented extensively in the scientific literature.[1−7] These experimental results are of central importance for testing and refining contemporary theories regarding the chemical origins of life.[8,9] Astronomical observations and simulations of interstellar chemistry have also revealed that key precursors of biological molecules are widespread in the universe.[10,11] A discussion of such experiments and observations in the classroom typically generates a great deal of interest among students. However, performance of model prebiotic experiments can require expensive chemicals, hazardous gases, and complicated equipment,[12,13] making these experiments inaccessible at most high schools.

In this series of laboratory experiments, students carry out a model prebiotic reaction (with minimal hazards) that generates adenine, an essential component of DNA and RNA in all living organisms. They then extract DNA from a familiar source, the commercial strawberry, treat this DNA with heat to dissociate DNA-bound proteins, and then use heat and acid to release the purine nucleobases from the sugar−phosphate backbone of DNA. Using a robust and accessible analytical technique (thin-layer chromatography, TLC), the students are able to verify a model prebiotic reaction producing a central molecule of life. Many students readily appreciate the abiotic production of adenine from molecules that are widely distributed in the universe and understand how this reaction illustrates the deep connection that

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must exist between chemistry and biology, how such chemical reactions could occur widely across the universe, and how similar chemical reactions may have facilitated the emergence of life on earth.

The use of TLC to analyze chemical mixtures has been described.[14,15] A practical chromatography experiment is included in this series and can be completed by students for additional experience. Alternatively, TLC can be demonstrated prior to the first experiment of the series.

Many of the materials needed for the laboratory experiments can be purchased at grocery or discount stores and may already be available in the average high school lab. For other experimental supplies, cost has been considered. The materials list (see the Supporting Information) includes contact information for various suppliers and lower-cost alternatives for some materials that have been tested and shown to work in actual high school lab settings.

The experiments were developed for an introductory high school chemistry course, as well as for an Advanced Placement (AP) chemistry course, International Baccalaureate (IB) Diploma Program, or general chemistry undergraduate course. The prebiotic chemistry, DNA extraction, and TLC analysis experiments are consistent with the Georgia Performance Standards for high school chemistry courses,[16] the College Board Advanced Placement curriculum,[17] and the IB Chemistry Aims and Objectives.[18] The experiments can be used independently as their complete descriptions are available in Supporting Information and, except where otherwise indicated, do not have to be performed in the order listed but can be incorporated at points during the semester where they best complement the curriculum.

PROCEDURE AND RESULTS

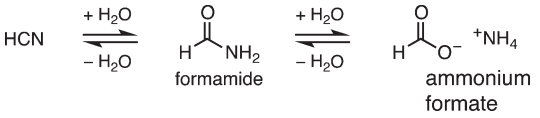
Demonstration

The four experiments presented here can be performed independently or as a four-part series. Experiment 1 introduces students to the concept of model prebiotic chemical reactions, experiment 2 teaches the students how to isolate DNA from a living organism, experiment 3 teaches the students how to release purine bases from DNA by the acid-catalyzed depurination reaction, and experiment 4 teaches the students about TLC. Adenine, produced chemically in experiment 1 and derived from a biological source in experiment 3, is easily identified by the use of TLC and authentic standards. Thus, the teacher may choose to conduct experiment 4 first as a means to introduce students to the TLC technique, so that students are able to use TLC in experiment 1 and experiment 3. However, if time is limited, the teacher may opt to demonstrate the TLC technique rather than having students perform the TLC procedure separately. If this option is selected, the correct setup and purpose of the various parts of a TLC chamber are described below in experiment 4 and in the Supporting Information.

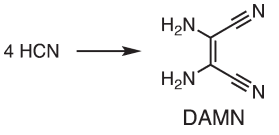
Experiment 1: Adenine Synthesis in a Model Prebiotic Reaction

Students use plausible prebiotic molecules to synthesize a nitrogenous base (adenine) that is found in the DNA of all living organisms. Hydrogen cyanide (or HCN) has long been considered a precursor to nitrogenous bases because HCN is a simple molecule that could have been produced in the atmosphere of the early earth.[2] One molecule of HCN will react with one molecule of water to produce formamide, also a likely prebiotic molecule (Scheme 1). Moreover, formamide can further react with water to produce ammonium formate, a salt (Scheme 1). The accepted relevance of HCN, formamide, and ammonium formate to prebiotic chemistry is also supported by the presence of water,[10,11,19] HCN,[11,20] and formamide[11,21] in interstellar space.

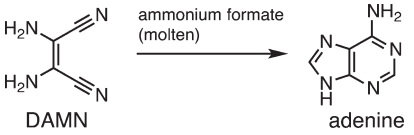
Scheme 1. Prebiotic Formation of Formamide and Ammonium Formate



Scheme 2. Prebiotic Formation of Diaminomaleonitrile (DAMN)



Scheme 3. Prebiotic Formation of Adenine



The molecule diaminomaleonitrile, or DAMN (Scheme 2), is also known as the HCN tetramer because it is made up of four hydrogen atoms, four carbons, and four nitrogens. DAMN is considered a potentially important molecule of prebiotic chemistry because it is an intermediate in the reaction pathway from concentrated HCN to adenine.[22] Inspiration for this experiment came from the discoveries that small quantities of adenine are formed in heated solutions of formamide[3,5,7] and that adenine is generated in good yield when DAMN is heated in molten ammonium formate (Scheme 3).[4] These reactions are considered relevant to understanding prebiotic chemistry and astrochemistry because formamide, ammonium formate, and DAMN are all products of HCN chemistry.[4]

To make this chemistry accessible to the high school classroom, a timesaving, simplified procedure for the synthesis of adenine in a model prebiotic reaction has been developed. Students perform a reaction in which ammonium formate is dissolved in formamide; DAMN is then added, and the reaction is heated to accomplish the formation of adenine. Heating may be carried out in an oven or a sand bath. The students compare, by TLC, the nitrogenous base synthesized in this reaction to authentic standards of adenine, thymine, and cytosine. Guanine is not used as a standard in these experiments because it has a low solubility in water.

Experiment 2: Extraction of DNA from Strawberries

Protocols for extracting DNA from different plants, fruits, and vegetables are available online,[23] but the goal of these various protocols is usually for students to visualize chromosomal DNA with the naked eye. A protocol is included for DNA extraction

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that allows students to use the extracted DNA in the hydrolysis reaction of an additional laboratory experiment in this series.

Strawberries are mashed in a DNA extraction buffer made of sodium chloride, clear liquid soap, and water. Frozen strawberries may also be thawed and used if fresh ones are not available; overripe strawberries are also acceptable. The strawberry mixture is then filtered using filter paper or cheesecloth to remove the solids before chilled ethanol is poured down the side of the container with the filtered strawberry mixture. This procedure causes the DNA to precipitate at the water/ethanol interface. Ninety-five percent ethanol (denatured) is preferred for this step, although 70% ethanol will also suffice. Concentrations of ethanol lower than 70% should not be used. The DNA obtained from this step is saved for use in experiment 3.

Experiment 3: Hydrolysis of DNA

This experiment consists of two parts, each designed to take one 45−60 min lab period. In the first part, students dissolve their extracted DNA from experiment 2 in water and heat this solution in a water bath to dissociate the DNA from histones and other bound proteins. The heated DNA solution is cooled, and 3 M hydrochloric acid is added; the solution is again heated in a water bath. Both heating steps are essential for liberated adenine to be detected using the TLC analysis protocol provided in experiment 4. The first, acid-free heating step must be performed separately from the acidic heating step. Adenine and guanine, the purine nucleobases of DNA (and RNA), become protonated in acidic solution, which facilitates the hydrolysis of the bond between these nucleobases and the sugar−phosphate backbone of DNA, a process known as depurination.

Part two of this experiment will need to be completed within one or two days of part one. In part two, TLC is used to analyze the partially hydrolyzed DNA. Because guanine has very low solubility in water, this base will remain at the origin line of the TLC plate. The adenine will migrate with the solvent up the plate and can be compared to authentic standards.

Experiment 4 (Optional): Thin-Layer Chromatography

This experiment is designed for teachers to implement in classrooms where they feel the students would benefit from extra practice with the TLC technique before moving on to experiment 1. Note that if experiment 4 is not performed, the time it takes to complete experiment 1 will likely be greater as students may need to gain proficiency with TLC. If the TLC demonstration option is chosen, in place of completing experiment 4, instructions for preparing and running a TLC plate are provided in experiment 1 as a reminder to students. However, if time permits, students may practice the TLC technique themselves by doing this experiment before beginning experiment 1.

Students are instructed in the basics of preparing TLC chambers and plates and calculation of retention factors (R_f). Students will run authentic standard solutions of adenine, thymine, and cytosine on their first TLC plate, and various mixtures of these compounds, prepared by the instructor, on their second TLC plate. They will then identify the unknowns from the mixtures based on their results with authentic standards.

A piece of filter paper is cut to dimensions that will fit into a plastic cup or beaker (the TLC chamber), reaching to the top, while covering one-half or less of the chamber wall surface area. This filter paper helps to saturate the inside of the chamber with solvent vapor. Photos are provided in the Supporting Information. Solvent, in this case, water, is placed in the chamber, taking care to keep the level below that of the origin line where the analyte spots are placed on the TLC plate. The chamber is covered with a watch glass or with plastic wrap and a rubber band.

The teacher also demonstrates TLC plate preparation and spotting. The origin line is marked with a pencil and ruler on the TLC plate, and marks are made where each analyte will be placed. Spots are carefully made on the plate using a glass capillary tube or flat toothpick. When dry, the plate is placed into the chamber and the water is allowed to travel most of the way up the plate, which is then removed from the chamber with forceps. The solvent front is marked. The plate is dried and then visualized with shortwave UV light (254 nm).

HAZARDS

Ultraviolet light is damaging to the skin and eyes. UV lamps, used for visualization of analytes on fluorescent TLC plates, should always face downward, and students should take care not to look directly into them. Caution should be exercised in handling hot containers after the model prebiotic reaction in experiment 1 and during the DNA hydrolysis in experiment 3. Glass capillary tubes used for TLC analyte spotting may be sharp, and students should not dispose of these tubes in the regular trash where cleaning staff may encounter them. (Glass capillary tubes may be placed into a secondary container, such as an empty plastic water bottle, before disposal.)

Because water is the eluting solvent, there are no hazards associated with the TLC developing chambers or with residual solvents on the TLC plates. Similarly, the DNA extraction buffer from experiment 2 contains safe ingredients and may be disposed of by pouring down the drain. Adenine, cytosine, and thymine are listed as irritants in crystalline form, but will only be used in very dilute solutions in this experiment series. If these solutions touch the skin, washing with soap and water is sufficient to remove nucleobases and prevent irritation. Formamide, ammonium formate, and DAMN are toxic by ingestion and inhalation, and therefore care should be taken to prevent contact of these chemicals with the skin or eyes. Students should wear gloves when handling these chemicals. Further, these chemicals should be disposed of in a designated organic waste container. Ethanol is a flammable solvent and an irritant. Hydrochloric acid, 3 M, is a strong acid and should be treated with extreme caution. Gloves should also be worn when handling HCl. Students should wear safety goggles at all times.

CONCLUSION

Experiments have been developed that allow prebiotic chemistry to be brought into the high school classroom. By participating in the series of low-cost laboratory experiments, using TLC as a means to identify compounds in biological and chemical samples, students gain a deep appreciation for the connections that exist between contemporary biochemistry (e.g., the molecular structure of DNA), molecules that are widespread in interstellar space, and how these components may have provided the chemical inventory that gave rise to biopolymers on the early Earth.

ASSOCIATED CONTENT

Supporting Information

Instructor preparation guide; student protocol and answer sheets; materials list (including part numbers and suggested suppliers); and additional safety information. This material is available via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Miller, S. L. *Science* **1953**, *117*, 528–529.
(2) Oró, J.; Kamat, S. S. *Nature* **1961**, *190*, 442–443.
(3) Saladino, R.; Crestini, C.; Costanzo, G.; Negri, R.; Di Mauro, E. *Bioorg. Med. Chem.* **2001**, *9*, 1249–1253.
(4) Hill, A.; Orgel, L. E. *Orig. Life Evol. B* **2002**, *32*, 99–102.
(5) Saladino, R.; Crestini, C.; Ciciriello, F.; Costanzo, G.; Di Mauro, E. *Chem. Biodiversity* **2007**, *4*, 694–720.
(6) Johnson, A. P.; Cleaves, H. J.; Dworkin, J. P.; Glavin, D. P.; Lazcano, A.; Bada, J. L. *Science* **2008**, *322*, 404–404.
(7) Barks, H. L.; Buckley, R.; Grieves, G. A.; DiMauro, E.; Hud, N. V.; Orlando, T. M. *ChemBioChem* **2010**, *11*, 1240–1243.
(8) Bada, J. L. *Earth Planet. Sci. Lett.* **2004**, *226*, 1–15.
(9) Lal, A. K. *Astrophys. Space Sci.* **2008**, *317*, 267–278.
(10) Bernstein, M. P.; Sandford, S. A.; Allamandola, L. J. *Sci. Am.* **1999**, *281*, 42–49.
(11) Hudson, R. L. *J. Chem. Educ.* **2006**, *83*, 1611–1616.
(12) Stong, C. L. *Sci. Am.* **1970**, *222*, 130–140.
(13) Navarro-Gonzalez, R.; Marambio-Dennett, E.; Castillo-Rojas, S. *Viva Origino* **1994**, *22*, 127–137.
(14) Dickson, H.; Kittredge, K. W.; Sarquis, A. M. *J. Chem. Educ.* **2004**, *81*, 1023–1025.
(15) Poole, C. F.; Poole, S. K. *Anal. Chem.* **1994**, *66*, 27A–37A.
(16) Georgia Performance Standards Homepage. https://www.georgiastandards.org/Standards/Pages/BrowseStandards/BrowseGPS.aspx (accessed Sep 2011).
(17) College Board AP: Chemistry. http://www.collegeboard.com/student/testing/ap/sub_chem.html?chem (accessed Sep 2011).
(18) International Baccalaureate Organization Diploma Programme Chemistry Guide. http://occ.ibo.org/ibis/documents/dp/gr4/chemistry/d_4_chemi_gui_0703_1_e.pdf (accessed Sep 2011).
(19) Cheung, A. C.; Rank, D. M.; Townes, C. H.; Thornton, D. D.; Welch, W. J. *Nature* **1969**, *221*, 626–628.
(20) Snyder, L. E.; Buhl, D. *Astrophys. J.* **1971**, *163*, L47–L52.
(21) Rubin, R. H.; G. W. Swenson, J.; Solomon, R. C.; Flygare, H. L. *Astrophys. J.* **1971**, L39–L44.
(22) Joyce, G. *Nature* **1989**, *338*, 217–224.
(23) How to extract DNA from anything living. http://learn.genetics.utah.edu/content/labs/extraction/howto/ (accessed Sep 2011).