How-to-do-it

Synthesis of Organic Molecules Under Simulated Prebiotic Earth Environments: A Simplified Approach

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According to the chemical evolution theory, life on earth arose spontaneously from inorganic matter about 3.6 to 4 billion years ago, under conditions far different from those present on the earth today, but according to normal chemical and physical laws. The events that led to the formation of life occurred when the inorganic molecules believed to have been present in the reducing primordial atmosphere reacted to form small organic molecules such as amino acids, sugars, and nitrogenous bases. The energy required for these reactions is thought to have come from solar ultraviolet light, electrical discharge (lightning), or perhaps even energy releases associated with volcanic activity. Once formed, these small organic molecules accumulated on the surface of the planet, where they interacted to form larger polymeric molecules, macromolecular aggregates, and then microscopically visible structured entities called "proteinoid microspheres" or "coacervates." Ultimately, these structured units evolved the characteristics that identified them as the first life forms to appear on the planet—"the protocell" (Oro 1980; Fox and Dose 1977). These events are summarized in Figure 1.

Laboratory exercises and demonstrations associated with classroom discussions of this theory traditionally include: (1) the identification of amino acids by chromatography; (2) the synthesis of polypeptides from amino acids; and (3) the formation of proteinoid microspheres or coacervates from proteins derived from natural sources (Vegotsky 1972; DeWitt and Brown 1977; DeToma and Campbell 1982). However, demonstrations of the initial step in the process, the abiotic formation of small organic molecules from inorganic gaseous molecules, are rarely attempted, because the required apparatus is not commercially available. Moreover, the apparatus is difficult and expensive to construct and to operate (Miller 1953; Stong 1979).

In this paper we describe the construction of a simple apparatus and a procedure to synthesize amino acids under conditions similar to those thought to have been present on the prebiotic earth. The protocol is based upon the classic experiment of Stanley Miller (1953), but uses a design that can be easily constructed. During the course of its operation, the system can be used as a motivational tool. Upon completion, sufficient product is available for students to assay for the presence of amino acids using paper or thin-layer chromatography.

Construction of the Apparatus

The Reaction Vessel:

The reaction vessel of the apparatus (Figure 2) is constructed from a 2-liter screw-top bottle of the type used by chemical supply houses to package acids and bases. Two 1 mm holes are carefully drilled into the walls of the bottle, on opposite sides, about 4 cm from the bottom using a carbide steel drill bit. Electrodes made from lengths of 1 mm diameter stainless steel wire are inserted into the newly-drilled holes and positioned so that they slightly overlap. The electrodes are then positioned to produce about a 0.5 cm gap between them. After positioning, the electrodes are secured with epoxy resin adhesive and the holes in the bottle are completely sealed with silicone sealant. The bottle is capped with a polypropylene screw-top into which inflow and outflow tubes have been inserted through holes drilled to accommodate them. All connections used in this apparatus are constructed of polypropylene tubing.

Gas Mixture:

There is no definitive evidence concerning the physical nature or chemical composition of the Earth's primordial "secondary" atmosphere. Thus, there is no consensus as to the gas mixture that should be used in experiments to demonstrate the prebiotic formation of organic molecules. A simulated prebiotic gas mixture of methane, ammonia, and water vapor

was chosen for this procedure because it is readily available and because it produces high yields of organic molecules. It is produced by passing natural gas from the laboratory gas supply through a concentrated solution of ammonium hydroxide (Miller 1982).

Electrical System:

The electrical system used to produce the required high voltage (20kv to 30kv) spark between the electrodes and simulate the electrical discharge (lightning of the prebiotic environment) is constructed from a low voltage (0-12v) D.C. power supply and a few readily available automotive parts. A 12v automobile ignition condenser and 12v heavy duty automobile signal flasher, wired in parallel, are connected between the positive terminal of the power supply and the positive primary pole of a 12v automotive ignition coil. The negative pole of the ignition coil is connected directly to the negative terminal of the power supply. The secondary positive pole of the ignition coil is then connected to one of the electrodes extending from the reaction vessel; the other electrode is grounded. Operation of the power supply at 8 to 9v is normally sufficient to activate the system and produce a strong, intermittent spark across the gap in the reaction vessel. After assembling the electrical components, it is best to test the spark gap. It may be necessary to bend the electrode wires in the reaction vessel so that a satisfactory spark is produced.

Condensation of intergalactic cloud of dust and gases to form the sun and planets of our solar system (about 4.6 billion years ago)

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Formation of the primordial "secondary" atmosphere of the Earth—the product of volcanic outgassing

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Production of small organic molecules from inorganic molecules present in the atmosphere

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Accumulation of small organic molecules on the surface of the Earth

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Polymerization of small organic molecules to form "macromolecules"

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Formation of macromolecular aggregates

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Macromolecules aggregated to form "proteinoid microspheres" or "coacervates"

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Earliest life forms (the protocell or protobiont) about 4 billion years ago

FIGURE 1. Outline of the events which, according to the chemical evolution theory led to the formation of life on earth (After Fox and Dose, 1977)

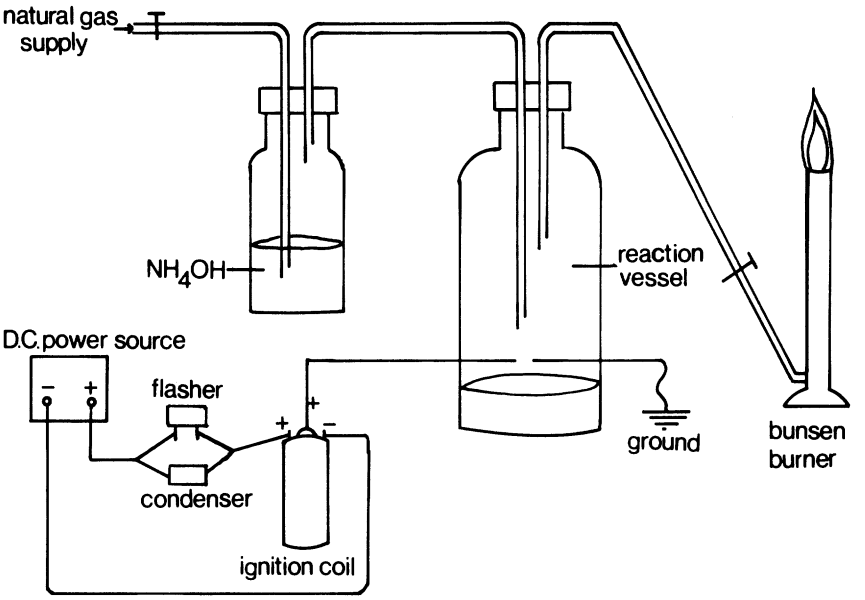

FIGURE 2. Schematic diagram of the apparatus used to synthesize organic molecules under simulated prebiotic earth conditions.

Operation of the System

Remember that this system employs high voltage and, if improperly assembled and operated, a flammable mixture of gases can result. It is, therefore, recommended that operation be restricted to experienced personnel taking all appropriate safety precautions. While the authors have had no mishaps, we recommend that the operator wear goggles and place the apparatus in a hood with a shatterproof transparent shield before activating the electrical system. In addition, observers should stay a safe distance away when activating the system.

To begin operation, add 50 ml of boiled distilled water to the reaction vessel. Place 300 ml of concentrated, reagent grade, ammonium hydroxide solution into the 500 ml wide-mouth, screw-top polyproplyene bottle which is connected between the natural gas supply and the reaction vessel. Close the system securely and seal all connections with silicone stopcock lubricant to prevent leaks. Turn on the natural gas supply and flush the system with the resulting methane-ammonia-water vapor gas mixture for at least 30 minutes to ensure removal of all oxygen. The excess gas mixture flowing out of the reaction vessel can be eliminated by burning in a Bunsen Burner. Note that, if the air intake of the Bunsen burner is closed and the system is adequately sealed, the flame produced will at first be blue and gradually turn yellow as the oxygen is eliminated. After flushing, the gas inflow and outflow tubes are clamped tightly shut and the high voltage system activated. It is recommended that the outflow tube be clamped first, in order to provide a slight positive pressure inside the reaction vessel. Operation of the system for a total of about 40 hours is sufficient to produce adequate product for analysis. During this time a tan to brown residue forms on the electrodes as well as on the walls of the reaction vessel. The water at the bot-

tom of the reaction vessel becomes colored with the material being formed, eventually turning a color resembling that of weak tea.

Analysis of Product

At the end of the operation period, the current is turned off. The water in the reaction vessel is swirled around to dissolve the materials accumulated on the electrodes and the vessel walls. The resulting "organic soup" is then prepared for analysis as follows (Miller 1953; DeToma 1982):

1. Evaporate the liquid to dryness in vacuo using a rotary evaporator with the flask immersed in boiling water bath. Elimination of water is achieved in about 30 minutes. The result is a film of brownish material which adheres to the walls of the flask.
2. Dissolve this residue in 3 ml of distilled water and make the resulting solution basic using several drops of a saturated solution of barium hydroxide. Litmus paper is sufficiently accurate to test for pH.
3. Evaporate to dryness as in Step 1 above.
4. Dissolve the residue in 3 ml of distilled water and acidify with 1 M sulfuric acid.
5. Evaporate to dryness as in Step 1 and then resuspend in 2 ml of distilled water. Add sufficient barium hydroxide to neutralize the solution.
6. Centrifuge the material and retain the liquid portion. Discard the insoluble precipitate.
7. Reduce the volume of solution to 1 ml using the rotary evaporator.

Spot several drops of solution onto a piece of chromatography paper or a cellulose thin-layer chromatography plate and develop with a solvent of n-butanol:water:acetic acid (4:5:1). Spray the surface of the paper or plate with 1% ninhydrin solution dissolved in butanol and air dry. Then heat to 105 degrees C for five minutes to reveal the presence of amino acids. Care should be taken not to touch the chromatography plate with bare fingers. Fingerprints will contaminate the plates with biologically produced amino acids which will also be developed by the ninhydrin.

To date, this procedure has yielded at least four detectable amino acids, two of which have been tentatively identified as glycine and leucine. At least five additional amino acids, present in smaller amounts, have been detected and identified by HPLC analysis. It is also probable that other classes of organic molecules are synthesized in the procedure. The identification of these compounds could become a most interesting project for an advanced class.

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