Donovan, S., C. Stiefbold, and K. Sprague. 1996. Chemical properties of amino acids and identification of unknown amino acids. Pages 35–70, *in* Tested studies for laboratory teaching, Volume 17 (J. C. Glase, Editor). Proceedings of the 17th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 255 pages.

Contents

Introduction

Two labs, Chemical Properties of Amino Acids, and Identification of Unknown Amino Acids, were designed to illustrate the physical and chemical properties of amino acids that determine the shapes and biological activities of proteins. The labs are the first two in a series that is closely coordinated with lectures in Cellular Biochemistry—a sophomore level core curriculum course required of University of Oregon biology majors. The course is preceded by a course in Genetics and Evolution and one in Molecular Biology, and it is followed by a course in Cellular Physiology. This set of four courses, each with an associated laboratory course, presents students with the key principles and relationships that underlie all of biology.

The Cellular Biochemistry laboratory series was created originally by W.R. Sistrom. The two exercises presented here are new, and were created during a general revision whose goal was to focus students' intellectual effort on the lab exercise itself, instead of on the lab report. That is, we wanted students to be intellectually engaged while working on the lab—not only while writing the report sometime later. In the case of these two new labs, we also wanted to address specific aspects of amino acid structure and ionization that students consistently find difficult.

The method of presenting these lab exercises is as important as their content. We use a weekly lab lecture (1 hour) given to the full class of approximately 250 students to set the tone for each exercise by introducing the relevant concepts and techniques. The exercise itself is done during a three hour lab period in which 26-30 students and two teaching assistants participate. The lab work is structured to create a cooperative environment that encourages both questions from individuals and group work. The text of the lab manual is designed to provide continual intellectual challenge, rather than passive data collection.

Materials

Chemical Properties of Amino Acids

For each lab section (26-30 students):

A complete set of models of amino acids (CPK) plus spare parts
Demonstration of column chromatography equipment—including samples of column matrix that students can see and touch.

For each pair of students:

CPK space-filling components—enough to build glycine in three ionization states, plus alanine, isoleucine and serine in a single ionization state (pH 6)
- Nitrogen, tetrahedral (3)
- Nitrogen, trigonal (3)
- Carbon, tetrahedral (10)
- Carbon, trigonal (4)
- Hydrogen (31)
- Oxygen, double-bonded (8)
- Oxygen, single-bonded (5)

Identifying Unknown Amino Acids

For each lab section:

Demonstration of titration equipment
Equipment and materials for electrophoresis of amino acids
- Horizontal mini-gel apparatus, modified for paper electrophoresis of amino acids
- Electrophoresis paper, Whatman 3MM
- Stock solutions of amino acids
  - Valine (0.5M)
  - Arginine (0.5M)
  - Aspartate (0.5M)
  - Lysine (0.5M)
- P_{20} micropipettors
- Electrophoresis buffers
  - pH 2 (0.5M phosphoric acid)
  - pH 6 (0.5M sodium phosphate)
  - pH 11 (0.5M sodium carbonate)

Set of index cards, labeled from A to Q, to represent the complete set of possible unknowns. You will need enough sets of cards so that each pair of students (lab partners) will be able to draw six different unknowns.

Notes for Instructors

Lab Structure

Each lab exercise consists of three parts: a pre-lab assignment, a group of lab activities, and a brief report.

1. Pre-lab assignment The pre-lab assignment is used to focus students' thinking on the important concepts connected with the lab exercise. Instead of emphasizing technical issues, pre-lab assignments require students to construct a framework for understanding before coming to lab. Pre-lab exercises are due at the beginning of the lab period, and are checked and returned during the period. This procedure allows instructors to discover quickly what students don't understand, and to address those problems during the lab session.

2. Lab activities The lab exercise is divided into several distinct activities, each of which focuses on a particular concept. As part of each activity, students must answer questions that require specific predictions or applications of concepts. These questions (set in italics) are an important tool for monitoring understanding. Teaching assistants can use the questions to engage individual students in discussions of the lab material, and also to assess the progress of the group as a whole.

3. Lab report The lab report is short and focuses on applications of concepts learned during the lab. If there is time, students are encouraged to complete the lab report during the lab period. This often leads to group interactions and problem solving that can be monitored by the teaching assistants.

The Role of the Lab Instructors

It is important to recognize that this lab format demands very active participation by the lab instructors. We spend a great deal of time making sure that the teaching assistants understand the material, and training them to interact effectively with the students. The teaching assistants must be sufficiently confident to elicit questions from students and to probe comprehension. This involves circulating through the lab room, and engaging individual students in specific discussions that get at key ideas. These discussions should be encouraging, but they should also be very clear — so that students can recognize misconceptions, and instructors can identify common problems that should be discussed by the group as a whole.

Organizing the Logistics of the Unknown Amino Acids Activity

You will need to plan carefully to organize the flow of information and people during the unknown amino acids activity. Some students will be confused by the structure of the activity (e.g., index cards representing unknowns, experimental results collected from the instructor, interpreting test results).

When a group of students is ready (i.e. they have completed lab activities 1 through 5), they can pick up a group of unknowns from a teaching assistant. Before asking for test results (they are only allowed three results per unknown) they should think about their strategy for discriminating between the possible amino acids based on the tests they have at their disposal (titration, solubility, gel filtration, sulfur test, electrophoresis, formaldehyde derivitization, and presence of conjugated rings). After collecting each experimental result they should carefully narrow the list of possible amino acids for that unknown.