BIOCHEMISTRY AND MOLECULAR BIOLOGY EDUCATION
Vol. 34, No. 2, pp. 134–138, 2006

Problem-based Learning

Using Advanced Problems in Introductory Courses

SOME SAMPLE PROBLEMS AND WHY THEY WORK

Received for publication, November 18, 2005

Deborah Mowshowitz‡

From the Department of Biological Sciences, Columbia University, New York, New York 10027

This article presents several typical problems used in an introductory course in molecular biology and discusses why the problems are effective at increasing learning.

Keywords: Problem-based learning, problem solving.

BACKGROUND ON THE COURSE THAT USES THESE PROBLEMS

These problems were written for a course called "Introduction to Cell and Molecular Biology I." This is the first college course in biology taken by premedical students, biomedical engineers, and biology majors at Columbia University. The course has a prerequisite of general chemistry, and most of the students are sophomores or juniors. The course is narrower but deeper than most introductory courses; we have chosen to focus on a small number of topics in detail instead of doing a broad survey of many topics. The course is intermediate in level between the usual first year introductory biology course and the usual upper level undergraduate course in biochemistry, molecular biology, etc. When I started teaching this course, I could not find problems of the appropriate level of difficulty, so I wrote my own. All of the problems have been used by at least three generations of students, and most have been used by many more. For more information on the course, see our web site [1], which includes an extensive course description (syllabus), schedule, text books, and lecture notes.

ORIGIN AND USE OF THESE PROBLEMS

Most of the problems were originally written as exam questions but have been revised for study purposes. Over the years, my colleagues and I have amassed a wide range of problems that cover most of the major issues and expose most of the major mistakes that students tend to make. Most of these problems have been collected into a problem book that is produced by the local copy shop [2]. The book has been evolving for about 20 years and is currently in its 17th edition. The book has stayed pretty much the same for the last 10 years or so, but it is revised slightly every few years to make the problems clearer and to include new findings.

The questions in the problem book are assigned for homework but are not collected. Extensive answers to these problems are included in the book so that students can check their own work and learn from their mistakes. Required recitation (discussion) sections are held weekly so that students can get help reviewing the material and doing problems. (A weekly quiz is given to ensure attendance.) A small number of additional problems, without answers, are handed out and discussed in recitation each week, as suggested by Brian White [3]. The students work through these problems in small groups during the recitation, consulting with the teaching assistants as needed. These "recitation problems" have been very effective in raising the important issues and keeping both the students and the teaching assistants focused during recitation.

GOAL OF THESE PROBLEMS

These problems have one main goal: to help students develop a robust understanding of the material. They generally do not test simple recall. We and many others, for example, B. White [3], have observed repeatedly that students who claim to "understand the material" have memorized structures, pathways, etc. but do not understand (and cannot apply) the underlying principles. It is quite clear that from our point of view, they do *not* understand the material, although they have accurately memorized entire biochemical pathways. Problem solving seems to lead to a much better understanding of the material. We think the problems are effective because they force the students out of passive mode into active mode. The students are pushed to use the pathways, methods, and structures, etc. that they have studied. They can't just repeat back what they have memorized; they must figure out what piece of information is needed and then figure out how to apply it. As they engage actively with the material, a deeper level of understanding usually emerges.

NATURE OF THESE PROBLEMS

Most of these problems are based on experimental situations, usually real ones but occasionally invented. I

‡ To whom correspondence should be addressed: Dept. of Biological Sciences, Columbia University, MC 2453, 1212 Amsterdam Ave., New York, NY 10027. Tel.: 212-854-4497; Fax: 212-865-8246; E-mail: dbm2@columbia.edu.

agree with Harold White [4] that questions based on experiments are usually the most effective in developing student understanding and that it is easier and safer to use real experiments. (However, I sometimes find it useful to make up a situation to demonstrate a point.) I believe that experimental problems are effective for two reasons: researchers often do not realize what they take for granted, and students are often unaware of what they are missing. Doing problems about experimental situations helps students uncover what they don't know and helps instructors realize what they forgot to explain.

These problems are somewhat like Jozsef Szeberenyi's (see, for example, [5]) in that they require the analysis of experimental situations that are new to the students. The choices of answers to these questions are much less intricate than Szeberenyi's as I use the choices as a guide only and require my students to write out explanations. I believe that students learn more when they believe they will have to explain their answers. (This also allows me to figure out more easily what they did not understand.)

My problems are also like Szeberenyi's in that they start with simple questions (about a particular experimental set up) and gradually build up to more complex ones about the same scenario. However, my problems are different in that I don't usually ask my students to deal with real data or to know quite so much biology. The students generally don't have to analyze graphs, chromatograms, etc. as I usually summarize the results in a sentence or two and then ask about the implications. If they are given data, the data are usually relatively simple and/or idealized. I assume that students will learn to deal with the complexities of raw data in more advanced classes but that they will benefit from problem solving from the very beginning.

The problems below were designed for introductory classes for science majors, but similar problems have been used in courses at all levels, from undergraduate courses for non-scientists to graduate courses for Ph.D. students. All of these problems, like those written by Szeberenyi, force the students to deal with situations they have never encountered before. Therefore the answers cannot be memorized, nor can they be solved by algorithmic "plug and chug" methods.

SAMPLE PROBLEMS

Q-1. HbX is a variant of hemoglobin that has a single amino acid change in the α chain. HbA (normal) and HbX are not separable by native PAGE (gel electrophoresis without SDS).

A. The change from HbA to HbX could be:

   i) Leu → Asp

   ii) Asp → Ala

   iii) Leu → Lys

   iv) Lys → Ala

   v) Leu → Ala

   vi) Asp → Lys

B. Suppose that fingerprints of HbA have 25 spots and fingerprints of HbX prepared in the same way also have 25 spots. A fingerprint prepared from a mixture of HbA and HbX will probably have (<24) (24) (25) (26) (50) (>50) (can't predict how many) spots.

C. Should HbA and HbX be separable by SDS-PAGE?

Comment: I like this question because it helps the students get a better feel for both the molecules being discussed and the methods used to separate and characterize them. Many students think that a "spot" on a fingerprint must be an amino acid, not a peptide, and they do not realize that SDS-PAGE is not sensitive enough to detect a single amino acid change. Since they are told that migration on SDS-PAGE is a function of molecular weight, they assume that any difference at all in molecular weight, no matter how small a percentage, is detectable. This problem has another useful feature. It leads students to question whether the term α implies the presence of an α helix in a protein.

Q-2. You hydrolyze DNA from some ordinary bacteria. You don't break the DNA chains down completely; under the conditions you use, the DNA is broken at random into a mixture of mononucleotides and dinucleotides. In your hydrolysate (mixture of products), you have all possible combinations of mononucleotides (A, T, etc.) and dinucleotides (AT, GC, etc.).

A. If you measure the proportions of the various mononucleotides in your hydrolysate, you expect to find that the percentage of (A = G) (A = T) (A = U) (A = C) (A = U or T) (A = C or T) (none of these) (can't predict), *and*

B. If you measure the proportions of the various dinucleotides in your hydrolysate, you expect to find that the percentage of (AT = TA) (AA = TT) (AA = GG) (AT = AU) (AA = UU) (AT = TA and AA = TT) (none of these) (can't predict).

Comment: This question reviews the structure of DNA, and in particular, the consequences of its double-stranded, antiparallel nature. Part A is quite straightforward if you know all of the terms in the question. Part B requires some careful thought. I like this type of question as an alternative to asking about structure or vocabulary directly. This question always causes students to ask whether hydrolysis will break hydrogen bonds, which leads to a fruitful discussion about weak and strong bonds and the meaning of hydrolysis. (All of this may be obvious to any instructor, but it isn't at all obvious to many students, and you can't explain it to them until they see the problem themselves.) You can't answer part B unless you know that the two strands are antiparallel *and* you consider the consequences in this particular situation.

Q-3. Suppose that you grow cells mitotically in medium containing a radioactive precursor (P^*) that is incorporated only into DNA.

A. Are the cells eukaryotes or prokaryotes?

B. What radioactive precursor did you use?

C. Suppose that you grow the cells in medium containing the correct radioactive precursor (P*) for two generations, as follows:

Cells (Non-radioactive) $\xrightarrow{\text{Interphase + P*}}$ mitosis 1

Cells from mitosis 1 $\xrightarrow{\text{Interphase + P*}}$ mitosis 2

Then you look at chromosomes in metaphase of **mitosis 2** by autoradiography; you count the number of grains (radioactive emissions) over each chromatid.

C-1. Will you find radioactivity in every chromatid?

C-2. Will every radioactive chromatid be equally labeled (have the same number of grains over it)?

D. Suppose that you grow cells as in part C. The cells are from a haploid organism where $n = 2$.

D-1. How many chromatids will you find per cell at metaphase of **mitosis 2**?

D-2. Consider a single cell in metaphase of **mitosis 2**. If this single cell finishes mitosis, what is the chance that both daughter cells will contain equal amounts of radioactivity? Assume that all chromosomes are the same length.

Comment: This question covers the same material as J. Szeberenyi's recent question in BAMBED [5]; both are based on similar classic experiments. This problem helps students see how semiconservative DNA replication (described in prokaryotes) fits in with mitosis. This question is typical of our problems in several respects. First of all, it deliberately uses two easily confused technical terms, chromatid and chromosome. The student is not asked about definitions but must understand the differences in order to answer the question. Secondly, this question requires an understanding of a standard procedure (the use of radioactive tracers) that is taken for granted by most instructors and researchers but is not obvious at all to students. As a third feature, the problem combines two topics (semiconservative DNA replication and mitosis) that are often discussed separately.

Q-4. A. If 100 molecules of glucose are fermented, the net yield of ATP molecules should be approximately (100) (200) (300) (400) (can't predict how many; it depends on whether glucose is fermented to ethanol or lactate).

Note: Students are expected to do this question with a diagram of glycolysis in front of them. Also, this question refers to "glucose-phosphate" to keep the focus on energy/ATP yields and to avoid a discussion of the isomerization of glucose-phosphates in this context.

B. Glycogen (glucose-glucose $\cdots$) is broken down by the following reaction:

$$(\text{Glucose})_n + \text{phosphate} \rightarrow (\text{glucose})_{n-1} + \text{glucose-phosphate}$$

The reaction is repeated as often as necessary, and the glucose-phosphate is converted to glyceraldehyde-3-phosphate. The net result is:

(Reaction X) Glucose-phosphate + ATP $\rightarrow$ 2 glyceraldehyde-3-phosphate + ADP

The glyceraldehyde-3-phosphate then goes through fermentation (or glycolysis, etc.) as usual. Suppose that a molecule of glycogen, 100 glucose units long ($[\text{glucose}]_{100}$), is broken down to glucose-phosphate and then fermented. How many molecules of ATP will be formed? The net yield should be approximately (100) (200) (300) (400) molecules.

C. "Reaction X" shown above is actually the summary of many separate reactions. (Students are referred to their lecture notes, handouts, or texts.) Suppose that you wanted to covert glucose-phosphate to glyceraldehyde-3-phosphate *in vitro* (in a test tube). What would you have to add to your test tube, in addition to the components shown in reaction X?

D. Suppose that you try to ferment some glucose to ethanol *in vitro*. You add all of the enzymes of ethanol fermentation to your test tube, except that you forget to add the enzyme(s) for the conversion of pyruvate to ethanol + CO_2. You start with 10 mol of glucose and 0.001 mol of ATP and 0.01 mol of NAD^+ and 100 mol of ADP. The net yield of ATP should be (100) (20) (.01) (.02) (.005) mol.

Comment: This question asks about a pathway but does not ask the student to reproduce it. However, it tests whether the student understands what the pathway arrows mean. Many students have trouble following standard biochemical conventions and converting back and forth from standard chemical reactions to pathways; they confuse the roles of substrates, co-enzymes, enzymes, etc. This becomes obvious when the students have to start with an unusual compound or at an unusual point in the pathway, as in part B. This problem is designed to give the students practice in "reading" a pathway and understanding the roles of the various components.

ANSWERS TO THE PROBLEMS

Note: These answers are somewhat shorter and less detailed than the ones usually provided to the students.

Q-1 A. (e) Leu $\rightarrow$ Ala. This is the only change that does not change the charge of the protein. Native PAGE separates on the basis of charge.

B. 26 spots (each spot = 1 peptide, *not* 1 amino acid). HbA generates 25 spots. HbX generates 24 that are the same and one that is different.

C. No. The difference here in molecular weight is too small to be detected by SDS-PAGE.

Q-2. A. A = T.

B. AA = TT. These are the proportions in the original double-stranded molecules, and they will be the proportions found in the hydrolysate. AT does not equal TA because the strands are antiparallel. Many students have difficulty with this question because they think that there are only a few DNA molecules in the sample, and the results depend on where the molecules are broken. However, there are many, many DNA molecules in the starting sample, and each molecule is broken at ran-

dom, so that overall, the proportions of TT, AT, etc. in the hydrolysate will reflect the proportions in the original DNA.

Q-3. A. Eukaryotes (no mitosis in prokaryotes).

B. P* = thymidine or thymine. (Cells do not take up nucleotides.)

C-1. Yes; C-2. No. Every chromatid will be radioactive, but one chromatid in each chromosome will have about twice as much radioactivity; one chromatid will have label in both strands of the DNA, and the other chromatid will have label in only one strand.

D-1. Four chromatids. There are two chromosomes, and each has two chromatids.

D-2. One-half. The two homologous chromosomes can line up two ways at metaphase, relative to the metaphase plate. In one case, one daughter cell will get both doubly labeled chromatids, and the other will get both singly labeled chromatids. In the other case, each daughter cell will get one doubly labeled chromatid and one singly labeled one.

Q-4. A. 200. The ATP yield is the same whether glucose is fermented to ethanol or lactate.

B. 300. It takes about 100 ATP to break down the glycogen and convert it to 200 molecules of glyceraldehyde-3-phosphate. The glyceraldehyde-3-phosphate then feeds into glycolysis and is broken down to CO_2 and ethanol (or lactate). Each molecule of glyceraldehyde-3-phosphate broken down yields two molecules of ATP. The net yield of ATP molecules is $(2 \times 200) - 100 = 300$.

C. You would need to add the four enzymes for conversion of glucose-6-phosphate to glyceraldehyde-3-phosphate. You would also need ATP, as indicated. No NAD^+ or NADH is needed as it isn't involved in any of these reactions. (You would also need an additional enzyme for isomerization of glucose-phosphate. It isn't clear from the reactions shown, but breakdown of glycogen gives glucose-1-phosphate, and it must be converted to glucose-6-phosphate for use in glycolysis.)

D. 0.01. NAD^+ is needed for conversion of glyceraldehyde phosphate to diphosphoglyceric acid (see pathway). There is no way to reoxidize NADH, so fermentation will run only until all NAD^+ is reduced. It takes 2 mol of NAD^+ to ferment 1 mol of glucose with a yield of 2 mol of ATP per glucose. So stoichiometry is 1 mol of ATP made per NAD^+ reduced.

DISCUSSION

This report describes the use of advanced problems in an introductory molecular biology course for science students. How successful is this approach? I do not have any quantitative data, but I have the results of many years of course evaluations and much anecdotal evidence. There is some grumbling about the difficulty of the course, but it is counterbalanced by many positive comments from students about how much they have learned (and how well they did on the MCAT). The course gets one of the highest ratings in the department, and students have very positive things to say about the problems. Here are some typical quotes from the student evaluations:

The problems are very interesting and a very different way of doing bio than I had previously experienced.

The problem sets and reading material actually teach you something, regardless of the difficulty, and that's good!

I think the homework book was incredibly helpful though hard. Teaching us the actual applications is so important. The theory alone wouldn't make us scientists and doctors. Practicing the application in the classroom makes the class harder but more helpful.

Students write back from medical and graduate school that they find themselves better prepared than their peers, and many who work in laboratories report that they now, at last, understand what they are doing and can appreciate the discussions that take place around them. A small number of students are so intrigued by the material and the approach that they decide to go into research instead of medicine.

Many students, even very good ones, find biological problem solving hard at first because they are so used to memorizing and plugging into formulas. Therefore it is necessary to provide lots of guidance on how to solve experiment-based problems and a strong incentive to be sure the students do it properly. All of my classes have a weekly recitation (discussion) session run by teaching assistants to provide the necessary guidance. In addition, I answer E-mail queries and hold office hours. The incentive varies depending on the size of the class and the intrinsic motivation of the students. In some classes, we give out problems (without answers) that are collected and graded as homework. In other classes, in which the students are sufficiently self-motivated, we provide problems that have extensive answers so that the students can check their own work and learn from their mistakes as they go. In all classes, we provide a small number of additional problems (without answers) to be discussed in recitation with the help of the teaching assistants [3].

The students who use the sample problems described here are premedical students and science majors at Columbia University. These are highly selected students who can be expected to function at a high academic and scientific level. However, similar problems have been used successfully with high school students, non-science students at Columbia University, and non-science students elsewhere. I believe that almost any group of students can benefit from experiment-based problem solving as long as the difficulty of the problems is adjusted to fit the audience. The important thing is that the students are required to analyze a new situation. This forces students to use the material, not just to repeat it, and this drives the students toward a deeper and clearer understanding. The students are often unaware of what they do not

know, or what they are supposed to know, until they try to apply their knowledge. This sort of exercise is not just beneficial to students; it can also be very helpful to faculty. Scientists are often unaware of how much they think is obvious until they write problems and find out that students don't understand them. Solving problems helps the students learn, whereas writing problems helps the instructor teach.

REFERENCES

[1] Biology C2005/F2401: www.columbia.edu/cu/biology/courses/c2005/.
[2] D. Mowshowitz (2005) *Problems in Biochemistry, Genetics and Molecular Biology*, 17th Ed., revised, available from the author.
[3] B. White (1998) A curriculum for recitation sections in introductory biology, *J. Coll. Sci. Teach.* **27**, 407–410.
[4] H. White (1993) Research literature as a source of problems, *Biochem. Ed.* **21**, 205–207.
[5] J. Szeberenyi (2005) Analysis of DNA replication in plant cells, *Biochem. Mol. Biol. Educ.* **33**, 229–230.