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Protein Science — A Laboratory Course

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Introduction

Rarely do students at an undergraduate college have the opportunity to pursue intensive laboratory work. The *Interim* (a month-long term in January where the students take only one course) provides an ideal opportunity for a research-like experience. Students can devote a whole month to a single topic and carry out an intensive laboratory project.

Three years ago, a molecular biology concentration in biology or chemistry was added to the curriculum at St Olaf College. As part of this concentration, intensive lab-oriented Interim courses are being developed. During the first two years, a recombinant DNA course was offered by the authors.[1] This course involved the isolation and purification of a *Bacillus cereus* phospholipase C gene from an expression plasmid in an *Escherichia coli* host. The gene, once purified, was used to screen other bacterial genomes for similar genes. Students were involved in all aspects of the course and spent an average of 6–8 hours per day in lab.

During January, 1994, we offered a new laboratory-intensive Interim course entitled Protein Science. Our idea was to focus on a well-characterized protein and study it in some depth. We chose egg-white lysozyme since it has a well-defined catalytic function and a well-characterized three-dimensional structure, it is also easily isolated and quite stable. The course consisted of lectures on various aspects of protein structure and function, extensively illustrated by computer graphics, and a laboratory project. The latter included the isolation and purification of lysozyme, analysis of the purified protein by gel electrophoresis, and independent projects focused on its thermal stability.

Understanding protein folding is one of the major problems in molecular biology. Lysozyme, a small well-characterized monomeric protein, is a very attractive model for studying the folding problem.[2] Brian Matthews and his group at the University of Oregon are particularly noted for their work on bacteriophage T4 lysozyme.[3,4] Their approach has been to engineer site-directed mutants of T4 lysozyme and study the effects of amino acid substitution on protein stability by various physical techniques including X-ray crystallography. There is, thus, a large number of crystal structures of lysozyme available from the Brookhaven Protein Data Bank.[5] We have made good use of the extensive literature and X-ray crystal structure data on lysozyme in the Protein Science course.

Educational Objectives

Our primary goal for this course was to give students a research-like experience in a biochemistry/molecular biology lab. Semester-long courses usually have one 3–4 hour laboratory per week, where all the solutions are supplied and the project is designed to fit into the available time. This is not how science is done, but, due to the constraints of the students' schedule, it is often a necessity. Meeting only once a week, students lack a sense of continuity in what they are doing. The Interim provides this continuity and time to repeat experiments that may not work the first time. Protein Science was designed to allow the students sufficient time to be involved in all aspects of the course. This included literature searching, designing their own experiments, and preparing all the solutions. A secondary goal was to expose students to current ideas and technology of proteins. We accomplished this by having daily lectures and literature discussion prior to our laboratory work.

Organization of the Course

The class met for 1.5 hours each day for lecture and discussion. The remainder of the day was spent in the laboratory. We used *Introduction to Protein Structure* by Brandén and Tooze[6] as the lecture text. Kinemages, interactive protein graphics developed by Jane and David Richardson of Duke University,[7,8] were used to illustrate protein structure. The journal *Protein Science* uses kinemages to illustrate articles, and there is a kinemage supplement to the text, *Introduction to Protein Structure.* Students were encouraged to use computer graphics in their own presentations. The lecture topics are listed in Table 1. We began with lectures on protein structure, purification, function and thermodynamics, followed by individual presentations of current papers in the literature. The laboratory schedule is given in Table 2. The first two weeks focused on the isolation of lysozyme from chicken egg white. In the latter part of week 2 and week 3 the students designed and carried out their own exper-

Table 1 Lecture/Discussion Topics

Day	*Topic*
1	Protein characterization
2	Protein purification
3	Peptide structure
4, 5	Protein structure
6	Protein folding
7	Enzyme kinetics
8	Enzyme catalysis
9	Lysozyme structure and catalytic mechanism
10	Lysozyme stability, thermodynamics of unfolding
11–14	Literature presentations

Table 2 Laboratory Schedule

Day	*Experiment*
1	Organize lab, clean glassware, literature searching, write protocols
2	Protein assay, lysozyme activity assay, demonstrate computer graphics
3	Prepare solutions for lysozyme isolation, demonstrate column chromatography
4	Lysozyme isolation and CM-Sephadex chromatography
5	Assay column fractions (protein and enzyme activity)
6	Computer exercise — protein folding
7	Demonstrate electrophoresis, prepare solutions
8	Electrophoresis
9	Finish electrophoresis
10	Design projects, write protocols, prepare solutions
11–14	Work on projects
15	Field trip to protein laboratory, circular dichroism measurements (Mayo Clinic)
16–19	Prepare posters, clean up laboratory
20	Poster presentations

iments. The experiments all involved some aspects of the thermodynamics of lysozyme unfolding. On the last day of week 3 we visited a protein physical chemistry laboratory at the Mayo Clinic in Rochester, Minnesota, where we monitored lysozyme denaturation by circular dichroism spectroscopy. The final week was spent analyzing the data and preparing posters for presentation on the last day of class.

Kinemage and Computer Graphics

The kinemage ('kinetic image'), available through the Protein Society and the journal *Protein Science*, served as an innovative teaching tool in Protein Science. The computer program MAGE, developed by the Richardsons at Duke University for Macintosh and IBM/PC computers, illustrates protein structures in interactive and animated ways. The protein image can be rotated in real time and viewed in stereo. Kinemages are available for teaching and to illustrate articles in *Protein Science*. A program, PREKIN, is also available to construct kinemages from protein crystal structure data available from the Brookhaven Protein Data Bank.[5]

Kinemages are available through subscription to the journal *Protein Science*, on diskettes with each issue, and on CDs available to subscribers for the years 1992 and 1993. The kinemages and programs are also available free on the *Protein Science* FTP server on the Internet, by standard FTP, Gopher, and WWW Mosaic. By Gopher, send the command 'gopher ftp.uci.edu 1071'. This will place you at the University of California at Irvine's anonymous FTP directory. Select the item 'Protein Science Published by the Protein Society'. Files can easily be downloaded by FTP through Gopher. A kinemage supplement to *Introduction to Protein Structure*, containing the programs and an educational series of kinemages, is also available from Garland Publishing (Hamden, CT).

Equipment

Most of the equipment used in this course would be found in a typical biochemistry or cell biology laboratory. Some more specialized pieces of equipment were used and are specified as follows. We used a Bio-Rad Econo low-pressure chromatography system (BioRad no. 731–8101) for protein purification. Gel electrophoresis was done using Bio-Rad Mini-Protean II units. Thermal denaturation of lysozyme was followed on a Hewlett–Packard HP8452A diode array spectrophotometer with a Peltier temperature-controlled cell-holder, or a Perkin Elmer LS50B spectrofluorometer. Circular dichroism spectra were obtained on a Jasco J500A CD-spectrometer at the Mayo Foundation (Rochester, MN). None of the equipment specified above was critical and substitutions based on what is available at your institution are encouraged.

Kinemages were viewed in the classroom using a Macintosh computer, a Media Pro LCD projection panel (nView Corp., Newport News, VA) and a high-quality overhead projector. Students were able to work individually with kinemages on public computers, where the programs and kinemage files were available over the network server.

Experiments and Independent Projects

The course centered around an investigation of the thermodynamic properties of egg-white lysozyme. The students began by searching the literature for papers describing the isolation of lysozyme and its enzymatic assay.[9] They then wrote a protocol for lysozyme isolation and, after a demonstration of chromatography, performed the isolation. Following the CM-Sephadex chromatographic purification of lysozyme, all fractions were assayed for enzyme activity and the peak fractions were examined for purity by gel electrophoresis.

Students began independent projects at the end of the second week. After a discussion of the thermodynamics of protein folding and denaturation, the students were asked to design an experiment to study some aspect of lysozyme thermal stability. Projects included the effect of substrate binding on thermal stability, protein unfolding as a function of pH, and a comparison of fluorescence versus UV absorbance for monitoring thermal denaturation. All the students were involved in collecting circular dichroism spectra at temperatures through the melting region. These projects were completely designed and implemented by the students.

Thermal unfolding of egg-white lysozyme at low pH (1.6–3.0) can be followed by UV-difference spectroscopy.[10] The normalized transition curve is used to calculate K_D as a function of temperature, and then the thermodynamic parameters ΔG, ΔH, and ΔS. Figure 1

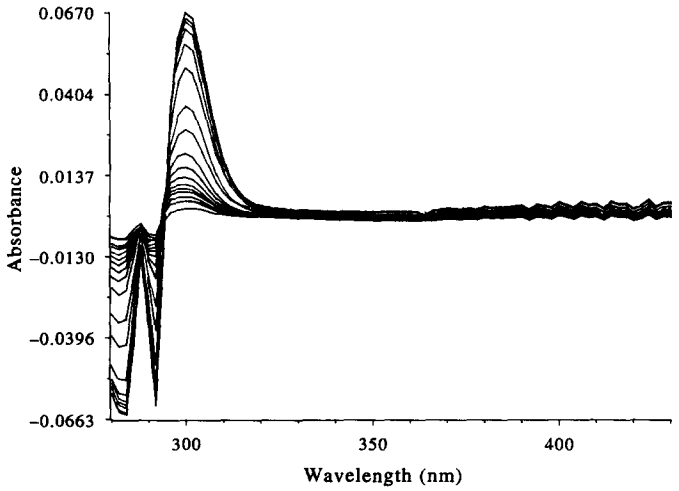

Figure 1 UV-Difference spectra of lysozyme at different temperatures. The spectrum of lysozyme (0.5 mg/ml, 0.1 M KCl, pH 2) at 20°C was recorded as the reference; the temperature was then increased, by 2°C each time, and the spectrum recorded as the sample

shows the UV-difference spectra at different temperatures, and Figure 2 shows the normalized transition curve. Upon lowering the temperature, folding was found to be completely reversible. Substrate stabilization of lysozyme to thermal denaturation can be studied in the presence of tri-*N*-acetylglucosamine (Sigma Chemical Co), an active-site inhibitor,[11] and unfolding can be studied as a function of pH.[12]

Unfolding can also be followed by fluorescence spectroscopy.[13] There is a significant linear temperature dependence of intrinsic tryptophan fluorescence in lysozyme,

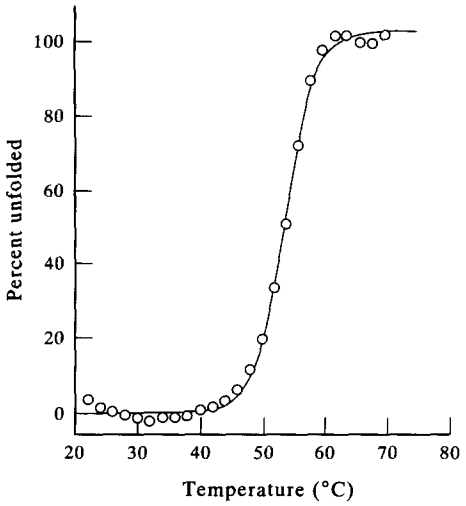

Figure 2 Normalized transition curve for lysozyme unfolding obtained from UV-difference spectra. Data from Fig 1 (ΔA_{300nm} versus temperature) were plotted, and the linear portions of the curve for completely folded and completely unfolded protein were subtracted; the resulting curve was normalized from zero to 100%. Data were fitted to a sigmoidal function (Logistic in SigmaPlot, Jandel Scientific, San Rafael, CA)

both in the folded and unfolded states, in addition to the change due to unfolding; these slopes have to be subtracted in order to obtain normalized transition curves. Figure 3 shows the fluorescence emission at 345 nm and the normalized transition curve.

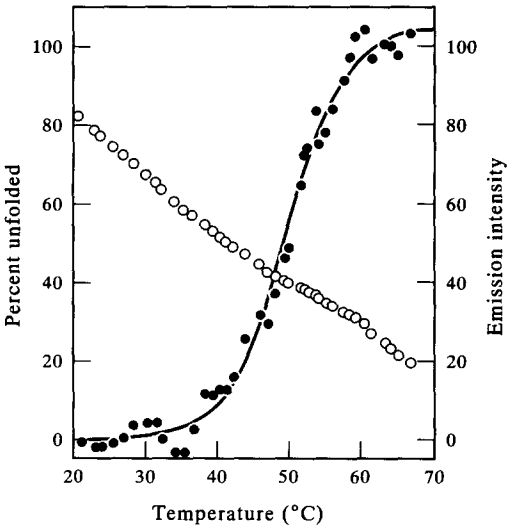

Figure 3 Lysozyme unfolding as followed by fluorescence spectroscopy. Lysozyme (0.3 mg/ml, 0.1 M KCl, pH 2.05) was excited at 290 nm and its emission measured at 345 nm as a function of temperature. The emission versus temperature curve (right ordinate, open circles) was corrected for the linear temperature-dependent increase in fluorescence of completely folded and completely unfolded protein, and normalized from zero to 100% to give the transition curve (left ordinate, closed circles)

Finally, thermal unfolding can be followed by changes in circular dichroism.[14,15] The large negative circular dichroism at 223 nm, due to α-helical structure, decreases to near zero as the protein unfolds. Figure 4 shows circular dichroism spectra at different temperatures through the melting region.

Writing and Posters

We required students to keep a detailed lab notebook of

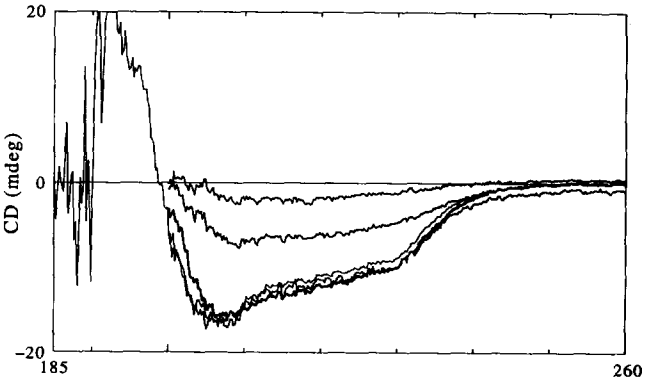

Figure 4 Circular dichroism spectra of lysozyme (0.5 mg/ml 0.1 M KCl, pH 1.65) at 15, 43.7, 49.3, 61.5, and 71.0°C

all the procedures performed. We checked the notebooks and made constructive comments when necessary. Oral progress reports were required every few days to guarantee the students were making progress and understanding what they were doing. During the final week of class, we instructed the students in the preparation of scientific posters. They then constructed posters and presented their projects at a poster session on the last day of class. Other faculty and students of the Biology and Chemistry Departments were invited to view the posters.

Grading and Evaluation

We evaluated the students on all aspects of the project. Lab notebooks, oral progress reports, posters, and lab participation were all graded. A final oral exam was required in which the students were tested on their comprehension of the principles they had learned and the projects they had completed. The student's enthusiasm and hard work in the lab had a major effect on the final grade.

Course Appraisal

This course involved close interactions between the students and instructors. The involvement of two instructors, one (HSH) from the Chemistry Department and one (JLG) from the Biology Department, made the course truly interdisciplinary. This course combined areas of expertise from both disciplines and showed the students that scientists in these two disciplines can cooperate in a significant way. The students felt a good sense of accomplishment, since they designed and carried out their own experiments. They liked the flexibility of working at their own pace in the lab, and having time to repeat experiments when necessary. They appreciated the fact that the responsibility for learning and working in the lab was largely theirs. They enjoyed the field trip to a first-class protein biochemistry lab at the Mayo Clinic, and were surprised that they felt quite comfortable in those surroundings after their experience at St Olaf.

Conclusions

The success of this course was due in large part to the Interim schedule and the opportunity of the students to spend successive full days in the lab. We designed the course so that students were involved in all aspects of their work. The amount of work required fit nicely into the available time. The choice of lysozyme as the focus for the course was good; it is a small protein and well characterized. Its thermal unfolding is easily followed by a variety of spectroscopic techniques, and the data obtained by the students were of high quality. In the future, we would like to include crystallization of the protein and a more detailed thermodynamic analysis of its unfolding. The use of computer graphics was an attractive feature of this course, and we would like to make more use of this in the future.

Acknowledgement

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