Concepts in Biochemistry

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Some Like It Cold: A Computer-Based Laboratory Introduction to Sequence and Tertiary Structure Comparison of Cold-Adapted Lactate Dehydrogenases Using Bioinformatics Tools

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The explosion of genomic sequence information made available to the public for data mining presents opportunities for comparative investigations of the structure and function of proteins and enzymes. Emerging biochemists of all levels will need the ability to efficiently make use of this growing body of information. In this computer-based laboratory experiment we introduce students to the free *user-friendly* bioinformatics tools developed by the San Diego Supercomputer Center (SDSC) at the Biology Workbench *(1)*. Students search for related protein sequences. The results of sequence alignments are easily downloaded or saved on a student-controlled account at the SDSC. Students can access the results of their work from any computer with Internet access. The Biology Workbench facilitates sequence and file formatting, which is more difficult in other sites. Procedures included with this exercise make comparison of sequence and tertiary structure readily accessible to a biochemist whose primary expertise is not computer-based.

The goals of this exercise are to reinforce the principles of protein stability, function, and temperature adaptation and to introduce students to resources available to them via the Internet. This exercise was developed to complement an interdisciplinary honors class exploring organisms' biochemical adaptations to temperature. Sequences of the enzyme lactate dehydrogenase (LDH) from related barracuda (genus *Sphyraena*) were downloaded and compared in order to isolate mechanisms of evolutionary adaptation to differences in growth temperature. Substitutions in amino acid sequences were located in a related crystallographic structure of LDH from the dogfish shark, *Squalus acanthias (2). Sphyraena idiastes, Sphyraena lucasana,* and *Sphyraena argentea* were chosen because of their close evolutionary relationship, different habitat temperatures, available kinetic data *(3),* and the availability of closely related crystal structures from *S. acanthias* for tertiary structure investigation. The close evolutionary relationship among the barracuda limits the number of amino acid substitutions in the LDH sequences and increases the likelihood that substitutions are related to temperature adaptation. Once students gained experience with the protein tools via examination of the published studies, they chose other sequences for novel comparisons.

The exercise can be carried out with Macintosh, Windows, or Unix platform computers with Internet access using available sequence databases at the National Library of Medicine *(4)* and protein structure database at the Research Collaboratory for Structural Bioinformatics (RCSB; ref *5*). Analysis of downloaded sequences and structures can be carried out with free access to Biology Workbench *(1)*, and freeware molecular visualization with Rasmol *(6)* or Protein Explorer *(7)* and the Molecular Visualization Freeware site maintained by Eric Martz *(8)*.

This laboratory is appropriate for biochemistry and molecular biology laboratory courses, special topics, and advanced biochemistry lecture courses. The exercise can also be adapted for honors high school programs. A background in protein structure, stability, and enzyme function should be prerequisite or provided in the course.

Procedural Overview

Prior to the laboratory, students are given a lecture on the types of amino acid substitutions expected in psychrophilic enzymes. Published reviews *(9–11)* cite a needed increase in protein flexibility and solubility at lower temperatures. Students also read a paper *(3)* containing thermal stability, sequence, and kinetic data from the three barracuda LDHs. In the three part exercise, students first access the NIH PubMed databases *(4)* and search for and download amino acid sequences of lactate dehydrogenases (LDH) from three closely related barracuda species from the genus *Sphyraena,* as well as *Squalus acanthias* and *Bacillus stearothermophilus (12).* Students then carry out sequence alignments using the "Protein Tools" and "Alignment Tools" at Biology Workbench. Finally, students download crystal structures and examine the barracuda amino acid substitutions in the context of the tertiary structure of the enzyme. Substitutions are then examined for correlation to cold-adaption in thermal stability and kinetic behavior.

Examination of the crystal structure of LDH isolated from *B. stearothermophilus* illustrates subunit contacts in the tetramer. Students are asked to identify the active site, locate the positions of the substitutions, and then classify each substitution as surface, interior, subunit contact, or active site. Because the dogfish (*S. acanthias*) is more closely related to *Sphyraena*, students also examine the crystal structure of the dogfish LDH monomer. Fewer amino acid substitutions exist

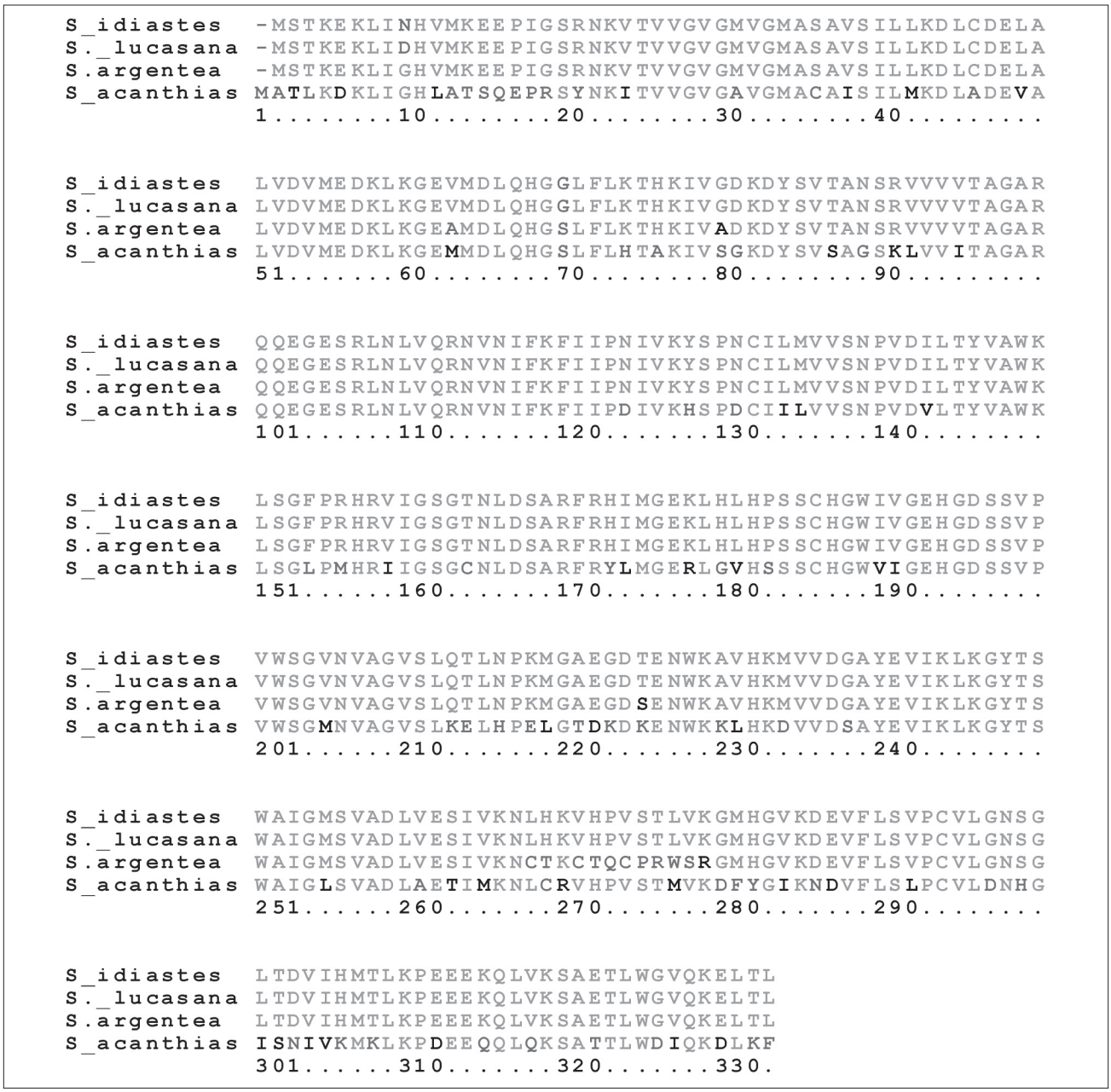

Figure 1. Sample sequence alignment of LDH from *Sphyraena lucasana*, *Sphyraena idiastes*, *Sphyraena argentea*, and *Squalus acanthias* carried out by students in the computer laboratory. Black letters denote homologous substitution and dark gray letters denote a non-homologous substitution. Comparison of protein amino acid sequences of LDH of closely related fish within a genus limits the number of substitutions and facilitates correlation of substitutions to temperature adaptation.

between the dogfish and barracuda LDH sequences, thus facilitating consideration of the effects of specific amino acid substitutions on tertiary structure. Students also are asked to classify the substitutions, if possible, into the categories (included in Instructor Materials in the Supplemental Materials[W]) of typical substitutions in temperature adaptation.

Once students are familiarized with the procedures for obtaining and comparing sequence and structure, small groups of students chose closely related bacterial species adapted to thermophilic or psychrophilic conditions for novel comparisons of their enzymes. Students examine the structures and tabulate amino acid substitutions consistent with temperature adaptation.

Conclusions

Students found this exercise extremely challenging but valued the deeper insights to protein structure and function that they were able to gain. A typical sequence alignment is shown in Figure 1. We offered several hands-on, in-class

sessions on the computers to help the students through the software aspects of the project. Carrying out their own sequence alignment greatly increased their understanding of what the plots actually represent. Student insights of amino acid structure and chemical properties became more sophisticated through analysis of the structures. Independent investigation facilitated a higher level of analysis of protein structure and function, a skill not easily taught in a passive lecture where structures are displayed and rotated by the instructor.

Students observed through comparison of the *B. stearothermophilus* and *S. acanthias* sequences and structures that tertiary structure is more highly conserved than primary sequence. Additionally, no amino acid substitutions occurred in positions that would dramatically alter the active site geometry. Amino acid substitutions primarily occurred on the surface of the enzyme and in locations near subunit interfaces. A more detailed analysis of substitutions and their classifications is included in the Instructor Materials.[W]

In their independent projects students found that *even within a genus*, there are very large numbers of amino acid substitutions and not all of them likely to be temperature adaptive. The best comparative investigations will be within a genus and as closely related as possible to identify adaptive changes that relate to function and stability. Future studies may include examples of laboratory evolved enzymes that incorporate fewer substitutions and enhance both catalytic and thermostability properties of the enzymes.

Although we chose to examine temperature adaptation, this laboratory is a template for a multitude of potential exercises. Instructors may instead choose to focus on catalytic mechanism or to combine this exercise with other approaches published in this *Journal (13–15).* Substrate bound crystallographic structures are available for LDH for both the *B. stearothermophilus* (1LDN.pdb) and the *S. acanthias* enzyme (3LDH.pdb), as well as its apo form (6LDH.pdb). For comparison purposes, an overlay of the free LDH enzyme and the NAD^+:pyrvate:enzyme ternary complex was constructed to illustrate the movement of the loop during catalysis. These structures have been compared in the literature *(16).*

Hazards

There are no hazards associated with the laboratory.

[W]Supplemental Material

Instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

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