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From Goat's Milk to Protein Structure: An Inquiry – based Case Study on Antithrombin III

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Introduction

Students are often excited to hear in the news about how recombinant proteins are being used as new treatments for diseases, but paradoxically they are less excited to memorize the endless details of complex protein purification procedures and minutiae of protein structure that are commonly presented in introductory biochemistry courses. Most students appreciate the value and relevance of understanding protein purification and protein structure only after they learn in more detail about different aspects of biochemistry and can see for themselves the central importance of these basic concepts. To overcome this pedagogical challenge, a case study has been developed to help students better appreciate the centrality and relevance of protein purification and protein structure within a biochemical microcosm. The case study explores how basic concepts of protein purification and protein structure are intertwined with the function and practical applications of recombinant antithrombin III produced in goat's milk. It is based on an interactive and inquiry-based computer-based laboratory exercise and has been successfully implemented in a large (~500 students) introductory biochemistry course taught at the second-year undergraduate level.

The case study guides students through a self-directed investigation of the basic biochemical properties and molecular structure of antithrombin III. Students are presented with some background information on the medical importance of antithrombin and the biotechnological basis for the production and purification of therapeutically useful amounts of recombinant antithrombin. These practical and real-life ideas are related to basic principles of protein structure and chemistry through the use of interactive exercises using PyMOL (Schrödinger LLC) and a newly developed Java Applet written by the author. The PyMOL-based exercises lead students to investigate how the three-dimensional, folded structure of antithrombin relates to the function of the protein as a proteinase inhibitor, as well as how the distribution of charged residues on its surface affect the binding of the protein to chromatography resins as a part of the purification procedure. The exercises developed around the Java Applet Ion Exchange Chromatography-Simulator (IECS) allow students to adjust the pH of the column chromatography buffer, as well as the isoelectric points (pI) of two proteins to explore how the rate of migration through the column varies with these parameters (Fig. 1). Sliders are incorporated into the applet to provide students with an intuitive, real-time interface for varying key parameters and observing their effects on the separation and hence purification of proteins. Similar exercise to those used by students in the introductory biochemistry course in which the author teaches can be accessed through the following website:

http://people.ucalgary.ca/~mibarret/able2010/mini1/at1.html

Students who have used these exercises following classroom lectures on protein structure and protein purification have provided very positive feedback on the utility of this case study. Most students indicate a lack of interest in learning about protein structure and protein purification before the relevance of these abstract concepts and procedures were clarified through the case study. By engaging students in a positive and interactive manner, this case study provides students with a current and innovative example that shows how abstract concepts central to the introductory biochemistry curriculum relate to real-life applications related to their long-term career interests in the biomedical sciences. The further development of this case study and others, as well as the integration of software tools such as PyMOL and IECS into active-learning exercises, is expected to provide a richer and more meaningful learning experience for students encountering many of the daunting details of introductory biochemistry.

Figure 1 (opposite page). Two screen shots of the IECS Java Applet showing how the simulation changes as the top slider bar (controlling the elution volume applied to the column) is dragged from the starting position at 10 mL to an ending position of 42 mL. The simulation demonstrates that the red protein (pI = 6.0, set by slider 2) migrates more slowly than the green protein (pI = 6.5, set by slider 3) on the Q-Sepharose anion exchange column, because the protein with the lower pI tends to have more negative charge and interacts more strongly with the positively charged Q-Sepharose column at pH = 8.0 (set by the bottom slider). The top panel shows the progress of the chromatography run after 10 mL of a linear sodium chloride gradient has been applied to the column, leading to a slight separation of the two proteins. The bottom panel shows that after 42 mL of the gradient has been applied, the green protein was eluted first (after 34 mL, as shown with the green chromatogram trace) and the red protein is just starting to elute (as shown by the red chromatogram trace and drops of red protein dripping from the bottom of the column).

Mission, Review Process & Disclaimer

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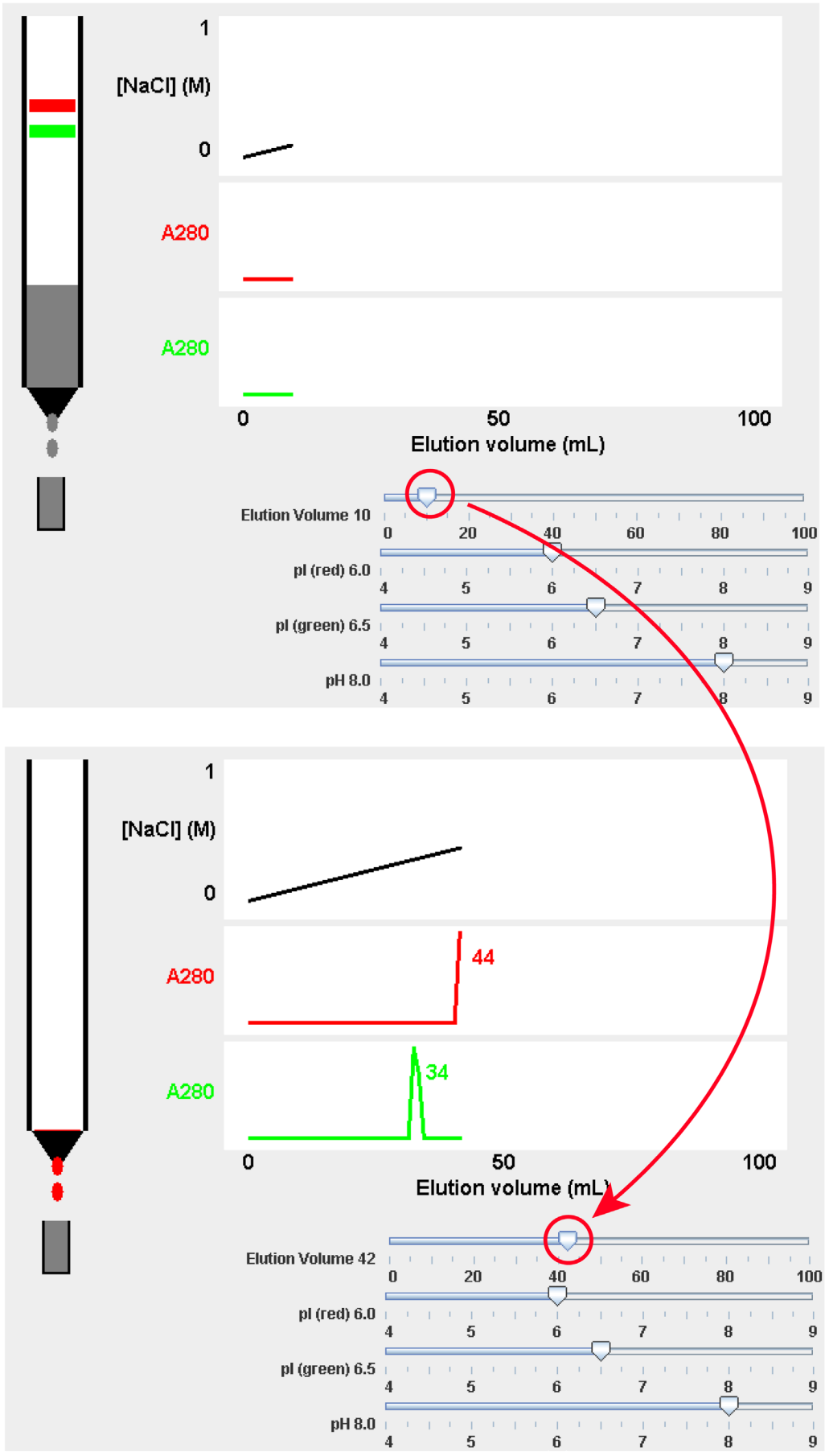
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A280
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50
100
Elution volume (mL)
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4 5 6 7 8 9
pI (green) 6.5
4 5 6 7 8 9
pH 8.0
4 5 6 7 8 9
1
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A280
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50
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Elution volume (mL)
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pI (green) 6.5
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pH 8.0
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