

**CHEM-342 Introduction to Biochemistry
Final Examination - Group (Part II)
Thursday, 28 May 2009
9:00 – 10:00 PM
H. B. White – Instructor**

Group Members

30 Points Total

Important - Please read this before you turn the page.

- You must sign your name on this page to receive the group grade. In case of lack of consensus, you may hand in your answer separately from your group for individual grading.
- You may refer to your notes, course reader, handouts, or graded homework assignments. Textbooks and reference books are not permitted.
- In CHEM-342, hemoglobin is a vehicle for learning how to learn by asking questions and pursuing answers to those questions. Undoubtedly you have learned a lot about hemoglobin in the process, but you also should be developing habits of mind that will enable you to solve problems in other courses and throughout your life. This part of the final examination provides an opportunity for you and the other members of your group to display problem-solving skills as a team. It is extremely unlikely that anyone in your group or in the class has encountered the information on the following pages. Your answers should display your collective:
 - breadth of knowledge (not limited to hemoglobin or biochemistry)
 - ability to analyze, make connections, and ask probing questions
 - sense of logic and organization
 - skill at generating models (testable hypotheses)

Biochemists study the chemistry of living things; however, most biochemists prefer to work *in vitro*, rather than *in vivo*. When one works with whole organisms, there are many uncontrolled variables that can prevent the sensible interpretation of results. Furthermore, if people or animals are involved, there are ethical considerations that justifiably can compromise or preclude a scientifically interesting *in vivo* experiment.

In vitro one can study biochemical phenomena in isolation with purified components and under controlled conditions where the effects of critical variables such as concentration, time, temperature, solvent, and pH can be measured quantitatively and systematically.

Sickle-cell disease is an *in vivo* medical problem. However, the phenomenon of sickling can be studied in whole blood *in vitro*. It is also possible to study the reversible aggregation process that causes sickling using pure solutions of hemoglobin S. The following is an abstract of an article that studied deoxyHbS aggregation *in vitro*.

J. Mol. Biol. (1977) **115**, 201-213

Kinetics of Hemoglobin S Gelation Followed by Continuously Sensitive Low-shear Viscosity Changes in Viscosity and Volume on Aggregation

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(Received 21 April 1977)

The kinetics of gelation of deoxyhemoglobin S were investigated as a function of temperature, concentration of hemoglobin, and solvent composition. Measurements were made by continuously monitoring the changes in viscosity with time, after polymerization had been induced by rapidly raising the temperature. A specially constructed low-shear viscometer was used. The solution density was also measured continuously to determine whether a volume change accompanied aggregation.

The results confirm earlier work in showing that the time-dependence of the viscosity is composed of a variable latent period (several minutes to tens of hours) during which there is only a slight and very gradual increase in viscosity, followed by a stage in which the viscosity rises very sharply within a very short time. The length of the initial latent period is highly dependent upon the HbS concentration ($33rd \pm 6$ power) and temperature. If the duration is interpreted as the inverse of a reaction rate, the activation energy is 96 ± 10 kcal/mol for solutions containing inositol hexaphosphate. Unlike measurements reported by others, no dependence of the latent period on shear rate was observed at the low shear rate employed. When IHP is omitted from the hemoglobin solutions, qualitatively similar results are obtained; however, the latent period depends on the $26th \pm 6$ power of the deoxyhemoglobin S concentration and yields an average activation energy of 125 ± 10 kcal/mol. The length of the latent period is increased 40-fold. Tris is known to prevent gelation but the inhibition can be partly reversed by adding IHP. When this is done, highly concentration dependent latent periods are again observed. The results may be interpreted in terms of nucleation kinetic theories: a critical nucleus composed of approximately 30 hemoglobin molecules is required for gelation; and the energy barrier (which is larger in the absence of IHP) to the formation of this critical aggregate is approximately 100 kcal/mol.

1. Based on the information in the abstract and the collective knowledge and resources of your group,
 - a. (5 points) Draw a figure with a title and legend that would represent the data from an experiment described in the above abstract. Label the axes. Try to generate a figure conveying the important information clearly and accurately. Use the back of this page if needed.

- b. (5 points) Draw a model of sickle cell hemoglobin polymerization that accommodates the information in the abstract.
- c. (5 points) I do not expect that you will fully understand this abstract. However, I do expect you to be able to generate a list of *at least 5* substantive learning issues. You may integrate information from the following abstract to enrich your learning issues.
- i.
 - ii.
 - iii.
 - iv.
 - v.
 - vi.
 - vii.
 - viii.
 - ix.
 - x.

2. Below are the abstract and two electron micrographs of deoxyHbS aggregates in two orientations from an article by McDade et al. (1989) *J. Molec. Biol.* **206**, 637-649.

On the Assembly of Sickle Hemoglobin Fascicles

WILLIAM A. MCDADE, BRIDGET CARRAGHER, CHRISTOPHER A. MILLER AND ROBERT JOSEPHS

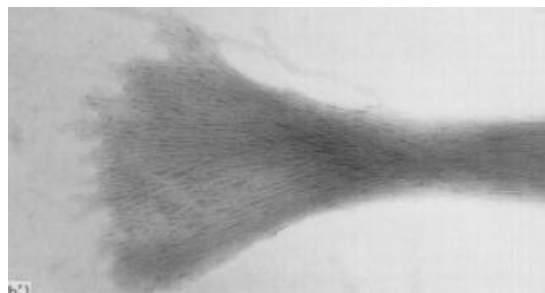
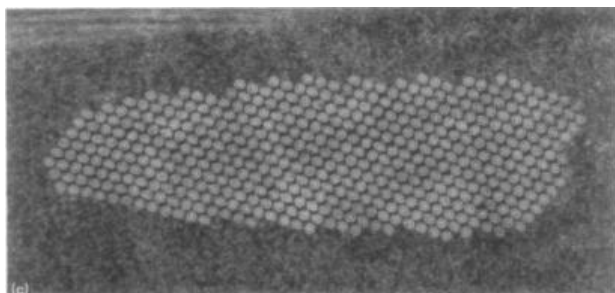
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(Received 24 April 1988, and in revised form 5 December 1988)

Deoxyhemoglobin S fibers associate into bundles, or fascicles, that subsequently crystallize by a process of alignment and fusion. We have used electron microscopy to study the formation of fascicles and the changes in fiber packing that occur during the conversion of fascicles to crystals.

The first event in crystallization involves fibers forming fascicles that are initially small and poorly ordered but, with time, become progressively larger and more highly ordered. After six to eight hours, the fibers in a fascicle form a crystalline lattice. The three dimensional unit cell parameters of this lattice are $a = 1300 \text{ \AA}$, $b=365 \text{ \AA}$, and $c=210 \text{ \AA}$ (the a axis is parallel to the fiber axis). Fibers have an elliptical cross-section whose major and minor axes are 250 \AA and 185 \AA respectively. When projected on to the unit cell vectors, these dimensions are 210 \AA and 155 \AA , so the unit cell dimension of 365 \AA implies that there are two fibers per unit cell. Theoretically, fibers could pair so that each member of the unit, cell is oriented in the same direction (parallel) or opposite directions (antiparallel). Fourier transforms of electron micrographs (or models) cannot distinguish between these alternatives, since the two arrangements produce very similar intensity distributions. The orientation of the fibers was determined from cross-sections of the fascicles in which the fibers are seen end-on. In this view the images of the fibers are rotationally blurred because the fibers twist 30° to 40° about their helical axis through the 300 \AA to 400 \AA thick section. We have been able to remove the rotational blur from each of the fibers in the unit cell using the procedures described by Carragher et al. The deblurred images of the two fibers in the unit cell are related by mirror symmetry. This relationship means that the fibers are antiparallel. These observations suggest that crystallization of fibers in fascicles is mediated by assembly of the fibers into antiparallel pairs that contain equal numbers of double strands running in each direction.



Electron microscopic pictures of polymerized deoxyHbS. The left view is of a cross section of fibers. The figure on the right is of a side view of deoxyHbS fibers.

- a. (5 points) Based on the information in the two abstracts and perhaps one of your learning issues, formulate a hypothesis that could lead to an experiment.

b. (5 points) Describe conceptually an experiment designed to test your hypothesis.

c. (5 points) What results would you expect from your experiment? (A figure of predicted results would save on words.)