Biology 5357

Chemistry & Physics of Biomolecules

Examination #3

Glycobiology, Membranes
& Membrane Proteins Module

January 2, 2021

Name: _____

Question 1. (15 points, 5 pts each)

(A) Draw a simple 2-D structure of the Glcβ-4Glc disaccharide, indicating the stereochemistry at each chiral center.

(B) What do the "β" and the "4" indicate in the name of this molecule. Indicate these on your structural drawing.

(C) This disaccharide adopts a specific conformation as part of the cellulose structure. If the ϕ angle is in the preferred conformation according to the anomeric effect, and there is a hydrogen bond between the hydroxyl group at position 3 of one residue and the ring oxygen of the other residue, then what is the approximate value of the ψ angle?

Question 2. (15 points, 5 pts each) Two of the current vaccines against the COVID-19 pandemic are ChAdOx1 (an adenovirial vector developed by Oxford and Astra Zenica) and mRNA-1273 (a full-length mRNA vector developed by Moderna). Both vaccines target the 1273 residue SARS-CoV-2 spike protein. This protein is N-glycosylated at some 22 Asn residues that are part of Asn-X-Ser/Thr sequences, where "X" can be any amino acid except Pro. At least 16 of these sites are clearly resolved by cryo-electron microscopy. Part of these glycans are high-mannose type and the rest are complex. Overall, the resulting glycan-shield covers an estimated 65% of the protein surface.

(A) From amino acid occurrence frequencies, does the spike protein appear to be unusually enriched in N-glycosylation sites?

(B) The glycans on glycoproteins of enveloped viruses like SARS-CoV-2 are produced in various compartments of the Golgi apparatus. This mechanism is sometimes said to be similar to an assembly line in an automobile plant. Explain.

(C) Briefly discuss the implications of the spike glycan-shield for potential vaccines against COVID-19.

Question 3. (20 points, 5 pts each) Read the attached paper from 1925, "On Bimolecular Layers of Lipoids on the Chromocytes of the Blood", by E. Gorter and F. Grendel, *The Journal of Experimental Medicine, **41***, 439 (1925).

(A) What is the hypothesis presented in the paper?

(B) Describe the experimental approach that is used to test this hypothesis.

(C) Identify three assumptions that the authors make when interpreting their experiments. Explain how these assumptions would lead to a change in the calculation of the membrane structure.

(D) With your current knowledge of membranes and membrane proteins, how would you alter your experimental approach if you were to repeat this experiment today– nearly 100 years later?

Question 4. (10 points) Cholesterol is an important component of biological membranes that can increase or decrease membrane fluidity under different conditions. Explain what these conditions are and how this happens considering the intermolecular interactions between neighboring lipids.

Question 5. (5 points) Describe four types of lipid bilayer mimetics that have been useful for purifying membrane proteins. What is an advantage or disadvantage of each?

Question 6. (20 points, 5 pts each) Consider ion channels and excitable membranes, and answer the following questions.

(A) Voltage-gated potassium channels possess a transmembrane helix that places approximately six arginines and lysines in the membrane. Consider the different amino acid hydrophobicity scales that were discussed in class. Which scale would be the most likely to predict that this helix is membrane embedded, and which scale would be the least? Explain the differences in the experimental approaches used to measure these two partitioning free energies and why one would prohibit partitioning, while the other may support it.

(B) Explain why potassium channels are selective for K^+ against Na^+.

(C) Explain why sodium channels are selective for Na^+ against K^+.

(D) During the cardiac action potential, the membrane potential depolarizes to about +40 mV before returning to the resting potential of about -90 mV. Explain why this happens and what ionic concentrations are required. Show your math!

Question 7. (10 points) Consider the three different types of transport activity: uniport, symport and antiport. What is common to each of these mechanisms? What features distinguish these three mechanisms? What are the driving forces for each type of transport? Describe an example for each.

Question 8. (5 points) In considering a molecular dynamics simulation, provide three issues for membrane protein simulations that are important and distinct from aqueous protein simulations.

ON BIMOLECULAR LAYERS OF LIPOIDS ON THE CHROMOCYTES OF THE BLOOD.

By E. GORTER, M.D., and F. GRENDEL.

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(Received for publication, December 15, 1924.)

We propose to demonstrate in this paper that the chromocytes of different animals are covered by a layer of lipoids just two molecules thick. If chromocytes are taken from an artery or vein, and are separated from the plasma by several washings with saline solution, and after that extracted with pure acetone in large amounts, one obtains a quantity of lipoids that is exactly sufficient to cover the total surface of the chromocytes in a layer that is two molecules thick. Subsequent extractions with ether or benzene yield only small traces of lipoid substances.

We therefore suppose that every chromocyte is surrounded by a layer of lipoids, of which the polar groups are directed to the inside and to the outside, in much the same way as Bragg (1) supposes the molecules to be orientated in a "crystal" of a fatty acid, and as the molecules of a soap bubble are according to Perrin (2). On the boundary of two phases, one being the watery solution of hemoglobin, and the other the plasma, such an orientation seems *a priori* to be the most probable one. Any other explanation that does not take account of this constant relation between the surface of the chromocytes and the content of lipoids seems very difficult to sustain.

Technique.

1. All the glassware (centrifuge tubes, pipettes, funnels, filters, beakers, extraction apparatus) were made fat-free by concentrated sulfuric acid to which potassium dichromate had been added.

2. The reagents (water, benzene, acetone, ether, etc.) were twice distilled in an all glass distillation apparatus. The salt was ignited before use in a quartz crucible.

3. The blood was taken directly from an artery or a vein. The vessel was laid free and a needle twice boiled in doubly distilled water to which first 1 per cent

soda, and then 0.5 per cent potassium oxalate had been added, was introduced into it. The first stream of blood was discarded to avoid the possibility of error from contamination with the fat of the subcutaneous tissue. The next portion was then permitted to flow into a small stoppered weighing bottle, containing 0.5 per cent potassium oxalate. In the case of the goat and the sheep the jugular vein was directly punctured through the skin but in this case the stream of blood was permitted to flow for some time, so as to wash the needle clean of all contaminating fatty substances before a measured quantity was received in our glass vessel. In human subjects the same procedure of puncturing the vein through the skin was followed.

4. After mixing, 10 cc. (or in later experiments 1 cc.) of blood were pipetted into a centrifuge tube of 60 cc. and three or four times washed with 50 cc. salt solution (0.9 per cent) in the usual way.

5. The extraction was performed with acetone during 48 or 72 hours. Large quantities were used.

After several extractions, the acetone was filtered into a glass beaker and the liquid evaporated on a water bath. This procedure was the most difficult part of the operation because loss was very liable to occur at this time. The residue was finally taken up in benzene and filtered into a measuring flask of 50 cc., when 10 cc. of the blood had been used, or in a tube marked at 2.5 or 5 cc., when 0.5 or 1 cc. had been taken. Just before each determination the liquid was made up to the mark with benzene.

Determination of the Surface Occupied by the Lipoids Spread Out in a Monomolecular Layer on Water.

Langmuir (3) has demonstrated that fats and fatty acids spread in a monomolecular layer when they have been dissolved in benzene and a few drops of the solution are placed on a large surface of water. Adam (4) has slightly modified the apparatus originally described by Langmuir. We have made use of Adam's modification. The benzene solution was delivered out of a calibrated 0.1 cc. pipette.

Now, it has been shown that the molecules of a fatty substance spreading on a water surface do not exert any pressure in a direction parallel to the surface before the condition is arrived at that they form precisely a monomolecular film, in which latter they come to be arranged in a vertical position. In the Langmuir-Adam apparatus the water surface chosen is so large that sufficient room is provided to the molecules so that they are not in close contact with each other. By the displacement of a strip of copper on which a balance is mounted one is able to determine the precise moment at which the molecules

begin to exert a pressure in a horizontal plane, and by placing different weights on the pan of the balance, it is possible to compensate and to measure this pressure. The reduction of the size of the surface is obtained by moving a glass strip covered with a thin layer of paraffin oil over the edges of the copper tray, which are covered as well with paraffin oil. As soon as the molecules are in close contact in a layer exactly one molecule thick, the balance moves out of the equilibrium position. By placing small weights on the balance one is able to compress the layer without much further reduction of the size of the surface, till suddenly by increasing the weight the layer is disturbed and equilibrium of the balance is no longer obtained. The dimensions of the surface are measured with a ruler.

We always began with the determination of the surface contamination. By placing 50 mg. in the pan of the balance and moving the glass strip from a distance of about 30 cm. we were able to determine that it hardly ever exceeded 0.5 cm. at room temperature.

From a pipette 0.1 cc. of the benzene solution of the lipoids of the chromocytes was blown onto the surface of the water in the tray and by moving the glass strip the point was noted at which the balance began to move, 50 mg. being the weight in the pan. The pressure exerted on each cm. of the layer was 2 dynes per 50 mg. weight in the pan.

Determination of the Number and the Dimensions of the Chromocytes.

The number of chromocytes was determined by filling the *mélangeur* as soon as possible from the weighing bottle containing the blood, and by counting in the counting chamber of Bürker the cells in 80 small squares, each measuring 1/4,000 c.mm. The surface of the chromocytes was evaluated from blood smears on slides, coloured by Pappenheim's panoptical dye. With the aid of a drawing prism of Zeiss 40 to 50 chromocytes were drawn on millimeter paper. By taking account of the magnifying power of the microscope one was able to measure the dimensions of the cells in a horizontal and a vertical direction.

The surface of the cells was derived from these numbers by making use of Knoll's (5) formula that in chromocytes having the form of a disc (a form that is taken by all chromocytes that are spread on glass) the surface is $2D^2$ (D being the diameter).

The total surface of the chromocytes from 1 to 10 cc. blood was easily obtained by multiplying the number of cells by their surface.

SUMMARY OF RESULTS.

We have examined the blood of man and of the rabbit, dog, guinea pig, sheep, and goat. There exists a great difference in the size of the red blood cells of these animals, but the total surfaces of the chro-

TABLE I.

	Animal.	Amount of blood used for the analysis.	No. of chromocytes per c.mm.	Surface of one chromocyte.	Total surface of the chromocytes (*a*).	Surface occupied by all the lipoids of the chromocytes (*b*).	Factor *a*:*b*.
		gm.		*sq. μ*	*sq. m.*	*sq. m.*	
1	Dog A	40	8,000,000	98	31.3	62	2
2		10	6,890,000	90	6.2	12.2	2
3	Sheep 1	10	9,900,000	29.8	2.95	6.2	2.1
4		9	9,900,000	29.8	2.65	5.8	2.2
5	Rabbit A	10	5,900,000	92.5	5.46	9.9	1.8
6		10	5,900,000	92.5	5.46	8.8	1.6
7		0.5	5,900,000	92.5	0.27	0.54	2
8	" B	1	6,600,000	74.4	0.49	0.96	2
9		10	6,600,000	74.4	4.9	9.8	2
10		10	6,600,000	74.4	4.9	9.8	2
11	Guinea Pig A	1	5,850,000	89.8	0.52	1.02	2
12		1	5,850,000	89.8	0.52	0.97	1.9
13	Goat 1	1	16,500,000	20.1	0.33	0.66	2
14		1	16,500,000	20.1	0.33	0.69	2.1
15		10	19,300,000	17.8	3.34	6.1	1.8
16		10	19,300,000	17.8	3.34	6.8	2
17		1	19,300,000	17.8	0.33	0.63	1.9
18	Man.	1	4,740,000	99.4	0.47	0.92	2
19		1	4,740,000	99.4	0.47	0.89	1.9

mocytes from 0.1 cc. blood do not show a similarly great divergence, because animals having very small cells (goat and sheep) have much greater quantities of these cells in their blood than animals with blood cells of larger dimensions (dog and rabbit).

We give all the results of our experiments, omitting only those in which we were unable to avoid losses in the procedure of evaporation of the acetone.

It is clear that all our results fit in well with the supposition that the chromocytes are covered by a layer of fatty substances that is two molecules thick.

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