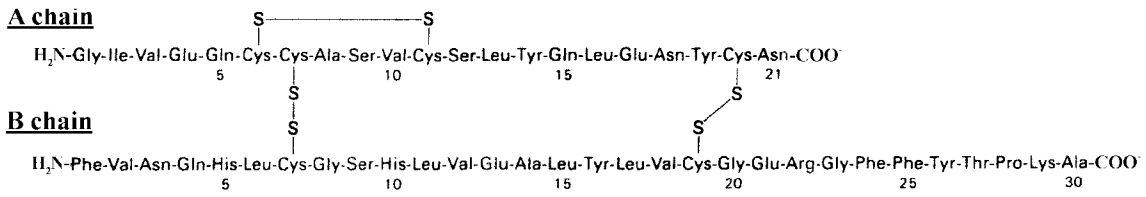
4. (26 pts) Consider the synthesis of insulin, a protein hormone with molecular weight of approximately 5.8 kDa which is secreted by endocrine cells of the pancreas in response to elevated blood glucose levels. The functional hormone consists of two subunits, "A" with a molecular weight of 2.3 kDa and "B" with a molecular weight of 3.5 kDa, linked by disulfhydryl bridges; the tertiary structure of the A chain is also maintained by an intramolecular disulhydryl linkage. The primary amino acid sequence of the subunits and the location of the disulfhydryl bridges is illustrated in the figure below.

Reduction of any of the three disulhydryl linkages inactivates the hormone; neither subunit by itself exhibits any insulin activity.

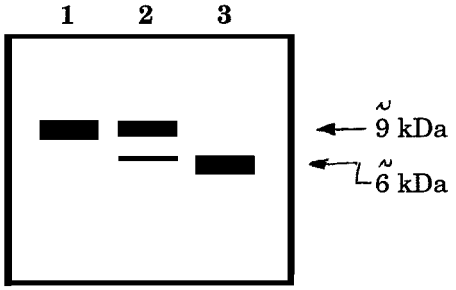


To examine the synthesis of insulin, homogenates of the endocrine gland were fractionated into organelle components and examined by SDS-PAGE (under oxydizing conditions that preserved the disulfhydryl linkages). The presence of insulin in each of these fractions was then determined using a radioactively labeled antibody specific for the hormone. An autoradiogram of the gel is reproduced below: each band represents the binding of the labeled antibody and a band's molecular weight is indicated in the right hand margin. (The technique is called a "Western" and the additional procedural details are irrelevant here.) Consider these data and what you know about protein synthesis, and answer all the following questions.

Lane 1: rough microsomes

Lane 2: Golgi vesicles

Lane 3: Secretory vesicles



A. (4 pts) Insulin is *not* a glycoprotein, although other Golgi-processed proteins produced by these glands are. How come?

Before (or after) you answer this question, consider the following *incorrect* answers. Several are not so much wrong, as *inappropriate*, because they address *why* insulin may have evolved the way it did and not *how* it's synthesized the way it is! Put another way, some answers provide an "ultimate" not a "proximal" reason for insulin to have the structure it does. They

are all quite imaginative answers, however! Some important features of the following answers to Question A. are *irrelevant* (beside the point) and - this should be the give-away – some rely heavily on information extraneous to BI250 text or lecture. Also, some of the information, relevant or not, is simply wrong!

Example 1: *Insulin is not a glycoprotein because it is secreted in response to elevated levels of glucose which means it must be stored in the cell prior to release. It is known that glycoproteins are not found in the cytoplasm and therefore stored insulin must not be glycosylated.*

Example 2: *Because the Golgi-apparatus is also responsible for the removal of glyco residues and due to the very nature of insulin. It breaks down saccharides it is unlikely to be glycosylated.*

Example 3: *Insulin is secreted as a "response to elevated blood glucose levels." If it were to have oligosaccharides attached to it, it would not lower the sugar concentration in blood to homeostasis as it is designed to because it would be adding sugars into the blood. As a result, disulfidryl bonds help form the tertiary structure instead of oligosaccharides.*

Example 4: *...Insulin may not need to be glycosylated to form its 2º, 3º, and 4º structure...*

What's wrong with the following answer? Use the space for your critique.

By looking at the molecular weights it appears that insulin possibly begins in the ER with a second (β) subunit. In the Golgi, however, rather thanm odifying the protein with sugars it removes a β-subunit and disulfide bonds become internal to the α-subunit. Other proteins are modified to become glycoproteins by the addition of sugars.

So what's a good answer? Here's an excellent one (from a student who ultimately earned an A+ for her final grade)!

It is likely not a glycoprotein because it does not have the correct amino acid sequences that would code for the addition of either an N-linked or O-linked oligosaccharide. For example it does not have the –Asp-X-Ser/Thr- sequence that codes for the addition of an N-linked oligosaccharide While many proteins are glycosylated in the Golgi complex, the process is not random, but proceeds in response to the information contained in the amino acid sequence (and ultimately in the nucleotide sequence of the gene).

B. (4 pts) Based on these data, is insulin the product of 1 or 2 genes? Why?

C. (8 pts) Describe the synthesis of insulin, using a diagram, that is consistent with these data and with your current understanding of protein synthesis.

D. (5 pts) Suggest an additional experiment that would test your hypothesized biosynthesis pathway and describe clearly what the results would indicate.

E. (5 pts) If insulin mRNA were translated in a test-tube, using a vesicle-free, SRP-free but otherwise complete cell-free translational system, how many different product(s) would be detected using the antibody described above. What would be its (their) molecular weights? **Why?**