Case 4
The Structure of Insulin
Last modified 3 January 2013

Focus concepts
The three dimensional structure of insulin is examined and sequences of various animal insulins are compared.

Prerequisites
- Amino acid structure
- Protein architecture
- Basic immunology

Background
Diabetics lack the protein insulin, which is produced by the pancreatic β-cells of the islets of Langerhans. Insulin stimulates uptake of glucose from the blood into the tissues. Diabetes is treated by replacing the missing insulin. Human insulin is produced industrially by recombinant bacteria, but before this method was available, animal insulin was used instead.

Insulin consists of two polypeptide chains, an A chain and a B chain, joined together by disulfide bonds. The smaller of the two chains is referred to as the A chain and is 21 amino acids long in humans. The second chain is referred to as the B chain and is 30 amino acids long in humans. Insulin from various animals is similar to, but not identical to human insulin, as illustrated in Table 4.1. A schematic diagram of the structure of insulin is shown in Figure 4.1.

Table 4.1: Variation in positions A8, A9, A10, B1, B2, B27 and B30 of insulin. (All other amino acids are the same.)

<table>
<thead>
<tr>
<th>Species</th>
<th>A8</th>
<th>A9</th>
<th>A10</th>
<th>B1</th>
<th>B2</th>
<th>B27</th>
<th>B30</th>
</tr>
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<tbody>
<tr>
<td>human</td>
<td>Thr</td>
<td>Ser</td>
<td>Ile</td>
<td>Phe</td>
<td>Val</td>
<td>Thr</td>
<td>Thr</td>
</tr>
<tr>
<td>cow</td>
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<td>Ser</td>
<td>Val</td>
<td>Phe</td>
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<td>Thr</td>
<td>Ala</td>
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</tr>
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<tr>
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<td>Pro</td>
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</tbody>
</table>
Questions
1. What animals would serve as the best sources of insulin to be used for treating diabetics? Explain your answer.

2. Would the \( pI \) values of the animal insulins be the same as, greater than, or less than human insulin?

3. Some people developed allergies to the animal insulin because their immune systems recognized the proteins as foreign. Explain why the immune system would be able to distinguish animal insulin from human insulin.

4. An SDS-PAGE gel is run of proinsulin and insulin. Samples were treated with beta-mercaptoethanol prior to electrophoresis. Draw a picture of the predicted results.

5. A denaturation/renaturation (similar to the one carried out by Anfinsen with ribonuclease) experiment was carried out using insulin. However, in contrast to Anfinsen's results, only less than 10% of the activity of insulin was recovered when urea and beta-mercaptoethanol were removed by dialysis. (This is the level of activity you would expect if the disulfide bridges paired randomly.) In contrast, if the experiment is repeated with proinsulin, full activity is restored upon renaturation. Explain these observations.

![Figure 4.1: The structure of proinsulin. The arrows show the cleavage sites of the peptide bonds that are hydrolyzed when proinsulin is converted to insulin (from Voet, Voet, and Pratt, 2013).](image)

6. Diabetics are treated with insulin, not proinsulin. Do you think that this is a good idea?
References
