Insulin preparations and analogues: structure and properties

Sara Sleigh

**ARTICLE POINTS**

1. Insulin is a protein hormone with a compact three-dimensional structure.
2. The insulin regimen of a person with diabetes aims to mimic as closely as possible a normal plasma insulin profile.
3. The properties of an insulin preparation determine whether it will be short or long acting.
4. Genetic engineering techniques enable new insulin preparations with advantageous properties to be produced.

**KEY WORDS**

- Insulin
- Properties
- Analogues
- Genetic engineering

**Introduction**

Insulin has been the focus of a great deal of scientific research during this century, owing to its biological and medical importance. In 1958, Fred Sanger from Cambridge University was awarded the Nobel prize for working out the amino acid sequence of insulin (Brown et al, 1955). This was a landmark in biochemistry as it proved that a protein has a precisely defined amino acid sequence. Insulin was also one of the first proteins for which the three-dimensional structure was determined.

**Insulin**

Insulin is a protein hormone whose primary functions are:

- To enable glucose to be transported across cell membranes
- To convert glucose into glycogen for storage in the liver and muscle
- To aid the conversion of excess glucose to fat
- To prevent the breakdown of protein for energy.

Insulin is manufactured in the beta cells of the islets of Langerhans in the pancreas. Initially, enzymes remove the N-terminal signal sequence from preproinsulin (a larger unfolded peptide chain) to give proinsulin (a precursor, single-chain inactive form). The connecting C-peptide is then removed from proinsulin by enzymes to give insulin (Figure 1).

At high concentrations insulin molecules associate in twos and, in the presence of zinc ions, sixes (forming dimers and hexamers respectively) (Figure 2). Insulin is stored in the pancreas as hexamers, ready to be released in response to external stimuli.

Proteins are made up of long chains of amino acids, which fold up to form stable molecules. There are 20 different naturally occurring amino acids. Each has an identical backbone which can be joined to another amino acid to form a chain, and a side-chain which determines the chemical nature of that amino acid (e.g. how large it is or whether or not it possesses a charge).

When the protein chain folds up, amino acid residues far apart from one another in the linear chain may be brought close together. This could result in the amino acids being perfectly aligned for enzymatic activity or, as in the case of insulin, being the correct shape and form to interact favourably with a complementary surface on another molecule — either another insulin molecule to form dimers or the insulin receptor (which is also a protein).

Insulin is formed from two chains of amino acids linked together chemically by disulphide bridges formed between the side-chains of four of these amino acids (Figure 3). The A chain contains 21 amino acids and the B chain 30. The two chains fold together to form the compact, three-dimensional insulin molecule (Figure 4).

The three-dimensional structure of a protein molecule may be analysed by passing X-rays through a crystal, which by...
definition contains a regular array of the protein molecules. The resulting pattern formed from the scattered X-ray beam can be analysed to give the exact positions of all the atoms in the crystal relative to one another (the technique is called X-ray crystallography).

The three-dimensional structure of insulin was determined in 1969 following years of pioneering work by Dorothy Crowfoot Hodgkin and her colleagues at Oxford University (Adams et al, 1969). It revealed a structure similar to that of other water-soluble proteins in which most of the amino acids that interact less favourably with water are located in the interior of the molecule, with the exception of two regions on the surface. These regions act as the 'glue' responsible for the formation of insulin into dimers and hexamers, and for its interaction with the insulin receptor. Two zinc ions are located at the centre of the hexamer.

This detailed knowledge of insulin’s three-dimensional structure has made it possible for researchers to investigate ways of changing its properties.

The need for new insulin preparations arises from the desire to mimic more closely in people with diabetes the normal physiological plasma insulin profile, while also minimising their discomfort and inconvenience (Figure 5). Insulin absorption into blood capillaries is the key to achieving this.

When the contents of an insulin granule are released from the pancreas into the bloodstream, the instant and massive dilution causes hexamers to break up quickly into active monomers (see Figure 2). However, the release of monomers from injected insulin hexamers and hence the action of this insulin occurs more slowly and depends critically on the following:

- The physical state of the preparation, i.e. soluble (clear preparation) or insoluble and particulate (cloudy preparation)
- The association properties of the insulin in the preparation, i.e. how readily the hexamers dissociate.

Mechanical factors such as massage, exercise and where and how the insulin is injected also affect the rate of insulin absorption. Indirectly, these affect the rate of dilution of the preparation and hence the dissociation of dimers and hexamers to produce active insulin.

The concentration and volume of insulin dose also play a role: a more concentrated dose of insulin injected in a smaller volume is absorbed more slowly than the same amount of insulin injected in a larger volume.

Plasma levels of insulin can also be affected by the binding of antibodies to insulin, causing insulin to be removed from the circulation, and by clearance from the bloodstream via the liver and kidneys (see Pickup and Williams 1997 for further details).
A number of different insulin preparations and analogues, whose characteristics depend on their physical and association states, are now available. These are discussed in detail below.

Insulin preparations
Preparations with different onset and action times can be manufactured by using additives to alter the solubility of preparations containing unmodified insulin.

Short-acting insulins
These insulins are used to cover the patient’s requirements for insulin at mealtimes. The preparations are clear in appearance as the insulin is in a soluble form, existing as an equilibrium mixture of monomers, dimers, tetramers, hexamers and higher association states (Brange et al, 1990).

Short-acting insulin is unmodified, stored at neutral pH and simply contains an additive (such as phenol or m-cresol) which prevents the growth of microorganisms in the preparation over time.

Delayed-action insulins
Delayed-action insulin preparations are used to reproduce the low level of background insulin produced normally (Figure 5).

The two main varieties of delayed-action insulins are isophane and lente insulins, both of which are insoluble suspensions of particles and hence appear cloudy. The absorption characteristics can be altered by varying the size of the particles of insulin in the suspension, larger particles being dispersed more slowly than small ones.

Isophane preparations contain equal concentrations of insulin, zinc ions and protamines. Protamines are small, highly basic proteins obtained from the sperm of salmon and trout. When added to solutions containing insulin at neutral pH, the protamine molecules bind to insulin and cause it to become insoluble. It should be noted that preparations from different manufacturers do not have the same absorption patterns, owing to variation in the size and shape of the insulin crystals.

Lente preparations, on the other hand, simply include excess zinc ions in an acetate buffer. This produces fairly insoluble zinc-insulin complexes, which can be prepared in two forms:
- Ultralente — a crystalline form in which the particles are large
- Semilente — an amorphous form with smaller particles that are absorbed more quickly.

Figure 3. The amino acid sequence of human insulin, and sites which participate in the formation of either dimers and hexamers, or both, and which are likely to interact with the insulin receptor (modified from Pickup and Williams, 1997).
Preparations containing 70% ultralente and 30% semilente insulin have similar absorption profiles to isophane insulin.

Both lente and isophane insulins suffer from variable absorption profiles because of differences in particle size — a problem that has been overcome in the delayed-action insulin analogues described below.

**Insulin analogues**

Using knowledge of the three-dimensional structure of insulin and biochemical data of its activity, researchers have been able to change specific amino acids in the sequence of the protein and hence subtly alter the properties of the insulin molecule (see Genetic engineering box).

Two entirely different approaches have been taken to produce fast- and slow-acting human insulin analogues, which can now be manufactured on a large scale.

**Fast-acting insulin analogues**

Examination of the crystal structure of insulin enables those amino acids specifically involved in dimer or hexamer formation, but not in receptor binding, to be identified. These can be changed using modern genetic engineering techniques (see box) and the characteristics of the resulting insulins studied.

For example, if crucial amino acids with small side-chains are replaced by amino acids with larger, bulky side-chains, dimer formation becomes a less favourable process.

Another approach is to replace a positively charged, or neutral amino acid, by a negatively charged amino acid, causing repulsion of the surfaces of two insulin molecules and thereby reducing the likelihood of dimer or hexamer formation. Thus, replacing the proline amino acid at B28 by aspartic acid results in the formation of an insulin analogue that exists essentially as a monomer (Brange et al, 1988).

Monomeric insulins are absorbed two to three times faster than rapid-acting insulin preparations and, since they act without the delay that would otherwise occur, they offer the great advantage that they can be administered with food.

**Delayed-action insulin analogues**

A number of different approaches have been applied to the problem of producing...
a soluble, slow-acting insulin analogue, preferably one that is absorbed slowly and steadily over 24 hours.

One approach has been to change the pH at which the insulin molecule is least soluble. This can be achieved by removing negatively charged carboxylate groups or by substituting positively charged lysine and arginine amino acids, thereby moving the point of least solubility from pH 5.4 to neutrality (around pH 7.0). These insulin analogues are prepared in slightly acidic solution, from which the insulin precipitates when injected as physiological pH is near neutral. This leads to more consistent absorption characteristics than are obtained with human ultralente insulin (Jørgensen et al, 1989).

A second, and totally different, approach has been to attach insulin to molecules such as fatty acids, which bind to albumin in subcutaneous tissue. Albumin is a non-specific binding protein and is known to have a significant influence on the rate of absorption of many drugs. Insulin is released slowly and evenly over a long period, closely mimicking background levels (Jensen-Hom et al, 1995).

Porcine and bovine insulins

The three-dimensional structures of porcine and bovine insulins are very similar to that of human insulin. However, porcine insulin differs from human insulin by a single amino acid and bovine insulin by three amino acids (Table 1).

Animal insulins have, in general, a slower onset and longer duration of action than human insulin, possibly because of an increased tendency to bind to fatty tissues, which slows absorption. Porcine insulin has less tendency to dissociate from hexamers compared with human insulin, although the clinical consequences of this may be negligible.

**Conclusion**

Research into insulin is ongoing, and the tools provided by modern molecular biology and genetics make the goal of reproducing a normal plasma insulin profile in a person with diabetes, while minimising their discomfort, ever more achievable. A body of research is also being undertaken into methods of administration of insulin, which will contribute to and hopefully improve the future treatment of people with diabetes.

---

**Table 1. Differences in the amino acid sequences of insulin from different species**

<table>
<thead>
<tr>
<th></th>
<th>A8</th>
<th>A10</th>
<th>B30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Threonine</td>
<td>Isoleucine</td>
<td>Threonine</td>
</tr>
<tr>
<td>Porcine</td>
<td>Threonine</td>
<td>Isoleucine</td>
<td>Alanine</td>
</tr>
<tr>
<td>Bovine</td>
<td>Alanine</td>
<td>Valine</td>
<td>Alanine</td>
</tr>
</tbody>
</table>

---


