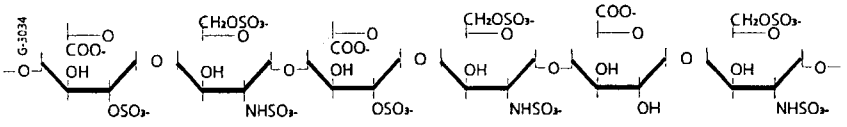
Use of Alcalase® in the Production of Heparin

Introduction

Heparin is an intracellular component of mast cells. These cells are widely distributed in animal tissue, including the mucosa of lungs and intestines from which heparin can be extracted.
A satisfactory yield of heparin necessitates decomposition of the surrounding tissues which can be achieved by using the proteolytic enzyme Alcalase.
Alcalase has no effect on the stability of heparin and improves filtration of the extract.

Heparin

Heparin is a mucopolysaccharide which inhibits coagulation of blood by potentiation of the activity of a plasma protease inhibitor, antithrombin III.
A complex of heparin and antithrombin III is a very effective inhibitor of several activated clotting enzymes, including factor Xa and thrombin.
Due to its anticoagulant activity, heparin is widely used to prevent thrombotic complications in surgery and during extracorporeal circulation. Furthermore, heparin is used to prevent extension of established thrombi.
The structure of heparin is the following:



The molecular weight ranges from 3,000 to 30,000 Daltons with an average molecular weight of around 15,000 Daltons. Heparin is normally available as a sodium or calcium salt.
Heparin is the most negatively charged molecule in animal tissues, a property which makes the purification of heparin fairly simple.

Alcalase

Alcalase 2.5 L, Type DX or Alcalase 3.0 T are proteolytic enzyme preparations produced by submerged fermentation of a selected strain of *Bacillus licheniformis.*
Detailed information about this enzyme is stated in Product Sheets B 259 and B 318, which are available on request.

Manufacture of Heparin

Today, heparin is almost exclusively produced from intestinal mucosa mainly from pork, but also from beef and sheep. Only minor quantities of heparin are produced from bovine lungs.

The following outline of heparin manufacture from porcine intestinal mucosa using Alcalase 2.5 L is meant only as an illustration of a possible process. In no way do the suggested dosages and processing times represent optimized process conditions.

a. Clean thoroughly the intestines with water.

b. Cut off the unnecessary parts like the pancreas, etc.

c. Pass the intestines through squeeze rollers to squeeze out the heparin-containing juices.

d. Adjust pH to 8.5 and heat denature by heating to 85-100°C and resting there for 10-30 minutes.

e. Cool down to 50-60°C.

f. Add Alcalase 2.5 L (1-5 g of Alcalase 2.5 L/kg of mucosa dependent on the reaction time). The tissue is digested for 4 to 24 hours (dependent on the amount of enzyme used).
The next steps depend on whether the heparin is going to be isolated by ion exchange chromatography or by precipitation with quaternary ammonium compounds. Ion exchange chromatography is generally preferred. If precipitation is used, a clear heparin-containing extract must be obtained, whereas ion exchange chromatography can be performed directly on the digested tissue or on a clear filtrate.

g.1. Clear filtrate:
Salt (e.g. sodium chloride, 2-5%), a flocculating agent (e.g. calcium chloride, 2-4%) and filter aid are added. The mixture is heated for 15 minutes at 90°C wherein the Alcalase is inactivated. The sludge is filtered off on a precoated filter press or drum filter. The heparin in the clear filtrate is adsorbed on an ion exchange resin or precipitated with quaternary ammonium compounds.

g.2. Digested tissue:
The digested tissue is filtered through a 50-mesh sieve. A macroporous, strong anion exchange resin (e.g. Lewatit MP500A, Bayer) is added and suspended in the mixture for 4 to 24 hours at 25 to 40°C and at pH 8 to 9.
After complete adsorption of heparin, the ion exchange resin is isolated and washed with a salt solution (e.g. 5% sodium chloride). The heparin is eluted by a concentrated salt solution (e.g. 20% sodium chloride).

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