B. Characterization of Insulin

I. Purity

1. Introduction

Proper characterization of the purity of insulin requires a combination of analytical methods based on various principles (Jørgensen et al. 1982). The four most important analytical principles for the characterization of insulin with respect to purity are the now classical biochemical methods of gel filtration (fractionation by molecular size) and disc electrophoresis (fractionation mainly by charge) to which have been added the highly sensitive methods of radioimmunoassay (RIA) and, recently, the high performance liquid chromatography (HPLC) methods. Improved modifications of HPLC techniques are expected to replace gel filtration and disc electrophoresis in the future.

2. Gel Filtration

In 1967 it was shown by means of gel filtration that commercial insulin, purified solely by crystallization, contained impurities with a higher molecular weight than that of insulin (Steiner 1967). These impurities were later identified to be mainly proinsulin, proinsulin intermediates and a covalent insulin dimer (Steiner et al. 1968).

Since then several gel filtration systems for the analysis of insulin have been described (Rolando and Torroba 1972, Schlichtkrull et al. 1974, Fisher and Porter 1981). Common to nearly all methods is the use of Bio-Gel P-30 (Bio-Rad Laboratories) or Sephadex G-50 (Pharmacia Fine Chemicals) and 1–3 M acetic acid as eluent. In this medium insulin is fully dissociated. Figure 4 shows three examples of gel filtration chromatograms of porcine insulin. In once crystallized insulin three distinct peaks are seen (a, b and c).

The a-component comprises high molecular weight substances (MW > 25,000) derived from pancreatic tissue proteins. Its concentration is reduced by crystallizations, but small amounts are still present in 5 times crystallized insulin, since antibodies against a-component are detectable in nearly all patients treated with insulin of this purity. (Heding et al. 1980).

The b-component contains proinsulin and related substances, which are removed to only a slight extent by crystallizations.

Finally, the c-component comprises insulin and derivatives of insulin with practically the same molecular size (insulin ethyl ester, arginine insulin, deamidated insulin, etc.) (Schlichtkrull et al. 1974).
Care should be taken when analysing insulin preparations by gel filtration to avoid possible misinterpretations due to the preservatives of the preparations (phenol, \textit{m}-cresol or methylparaben). These substances absorb UV light strongly at the wavelength used for detection of insulin (275–280 nm) resulting in the largest peak of the chromatogram. An example of such a misinterpretation has been described by Schlichtkrull (1977a). Insulin isolated from preparations still contains traces of preservatives which will lead to peaks in the chromatograms. As the preservatives are of low molecular weight and tend to adsorb to the gel, they are eluted after the insulin peaks.

Gel filtration of insulin using HPLC technique (cf. B.I.5) has been described by Welinder (1980).

3. Disc Electrophoresis

Since the introduction of disc electrophoresis in polyacrylamide gels by Ornstein (1964) and Davis (1964), and Mirsky and Kawamura’s (1966) subsequent application of this method for the analysis of insulin, it has been widely used for the characterization of insulin purity (Tjioe and Wacker 1972, Schlichtkrull et al. 1974, Krause and Beyer 1975, Kasama et al. 1980, Fisher and Porter 1981).

Although various electrophoretic systems are used by the different authors, they are all modifications of the original system by Davis and Ornstein operating at a slightly alkaline pH (8–9). Variations in acrylamide concentration, load per tube, content of dissociating agent (urea), and dye used for staining have been described. One of the most reproducible methods allows application of 100–200 μg of insulin per 5 × 50 mm tube containing 1 ml of gel (Schlichtkrull et al. 1974). At higher loads the insulin and monodesamido insulin are no longer distinguishable as two separate bands.