ISOELECTRIC FOCUSING OF PROINSULIN AND INTERMEDIATE IN POLYACRYLAMIDE GEL

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1. Introduction

Proinsulin and intermediates (of the conversion of proinsulin to insulin) have been found in serum as well as in pancreatic islets and were prepared from crystalline insulins of different species. Up to now the insulin precursors separated by gel filtration and ion exchange chromatography were studied with respect to homogeneity by electrophoresis only. To our knowledge, there are no experimental data on the isoelectric point of these proteins. In this paper we present evidence of the heterogeneity of proinsulin (PI) and intermediate (IM) after having used isoelectric focusing in polyacrylamide gel. The isoelectrically focused bands are characterized by both their isoelectric point (Ip) and their reaction with anti-insulin serum.

2. Materials and methods

Proinsulin and intermediate were prepared from crystalline bovine insulin (VEB Berlin-Chemie) as described [1, 2]. No impurities could be detected by immuno- and disc-electrophoresis. Only in disc-electrophoresis of proinsulin a faint band migrating towards the anode immediately before the major band was visible.

Acrylamide gels were prepared according to Conway-Jacobs and Lewin [3], merely, the volume of the solution was doubled and 200 µl of glycerol/tube were incorporated. The protein samples, 0.1 mg per gel in glass tubes 12 × 0.6 cm inner diameter, were electrofocused for 9 hr under cooling by circulating water up to 14°. 1% Phosphoric acid was used as the anolyte, and 1% ethylenediamine solution as the catholyte. A constant current of 0.67 mA per gel was applied until the voltage reached 310 V then this voltage was maintained [4]. The pH gradient in gels as well as Ip of the separated fractions were determinec at 22° by the method of Righetti and Drysdale [5]. Immunological experiments employed Catsimpoolas' sectional immuno-electrofocusing technique [6] using Ouchterlony plates (1% agar, Michaelis-buffer pH 8.2, ionic strength 0.01) and guinea pig anti-insulin serum.

3. Results and discussion

Proinsulin separated into two main bands numbered 1 and 2 (fig. 1) and three faint bands. The Ip of the bands 1 and 2 were estimated to be 5.52 ± 0.07 (number of electrofocusing experiments, n = 5) and 5.16 ± 0.07 (n = 4), respectively. Calculations of the Ip of bovine proinsulin [3] are in good agreement with the value estimated for fraction 1. The Ip of fraction 2 and its similarity to proinsulin in the immuno-precipitation with anti-insulin serum (fig. 2) indicates that this component probably represents a split proinsulin like that isolated from porcine insulin [7].

Fractions 4 (Ip 4.66 ± 0.11, n = 8) and 3 (Ip 4.81 ± 0.09, n = 9) most likely correspond to the intermediate forms lacking the Arg_{31}–Arg_{32} residues and the Lys_{59}–Arg_{60} sequence, respectively. These two intermediate forms could not be separated as yet. The Ip of components 2 and 1 were measured to be 4.90 ± 0.08 (n = 8) and 5.02 ± 0.09 (n = 9), respective-

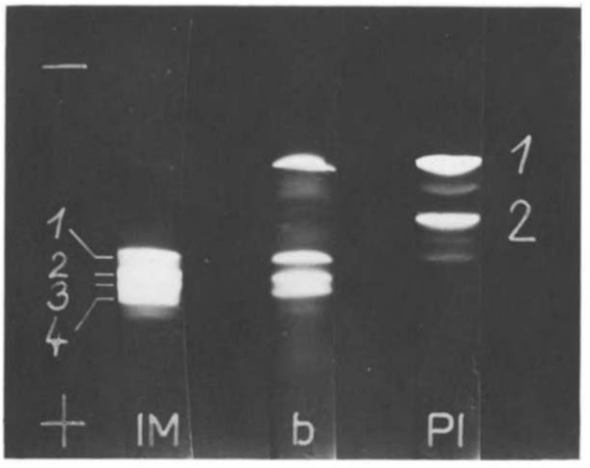

Fig. 1. Isoelectric fractionation of intermediate (IM), b-component (b), and proinsulin (PI) in 7.5% acrylamide gels containing 2% carrier ampholytes (pH 3–10).

ly, suggesting that in these intermediate forms only the residues Arg_{32} or Arg_{60} are cleaved out from the proinsulin molecule [8]. Further evidence supporting the existence of such closely related intermediates is the precipitation with insulin antibodies (fig. 3).

It is unknown whether these highly immunoreactive components in proinsulin and intermediate preparations are biosynthetic intermediates or not and further studies will be required particularly for the detection of endogenous intermediates. However, the method employed in this work may provide a suitable means for the investigation of intermediates in the transformation of proinsulin to insulin which have not yet been separable by conventional electrophoretic and chromatographic techniques.

Fig. 2. Gel diffusion of the focused fractions of proinsulin (PI) reacted with guinea pig anti-insulin serum. Numbers of the sample wells correspond to numbers pictured in fig. 1.

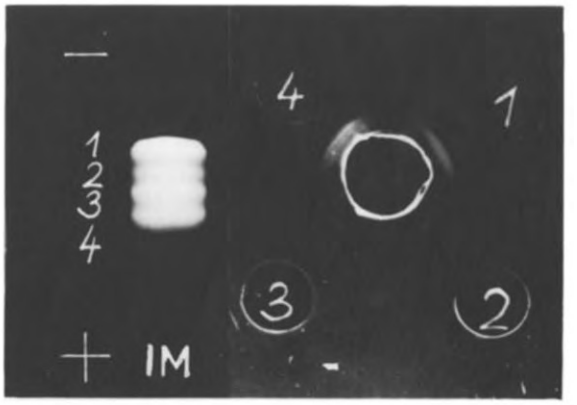

Fig. 3. Gel diffusion showing the immuno-precipitation of the fractions of IM and the focused components (right). Numbers refer to the fractions.

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