ISOELECTRIC ELECTROFOCUSING OF THE ENZYME OF YEAST ENOLASE

M. A. Geidan, M. R. Buka, and R. A. Zhagat

UDC 577.150.7:577.155.3

New prospects in the field of the study of proteins, enzymes, and their various molecular forms are opened up by the use of the method of isoelectric electrofocusing developed by Svensson and Vesterberg [1-3]. Isoelectric electrofocusing in polyacrylamide gel is a microanalytical modification of the above-mentioned method. A smooth gradient of the pH in gel electrofocusing is also created under the action of the electric field of ampholytes, but in this case the polyacrylamide gel acts as the stabilizing medium instead of a density gradient of sucrose. The method of electrofocusing in a gel is characterized by an extremely high resolving capacity and permits the simultaneous separation of 8-12 samples containing a total of several tens of micrograms of proteins in a short time.

Separation is performed in gel columns using an apparatus for disc electrophoresis [4-7] or electrophoresis in a thin layer of polyacrylamide gel [8]. Together with electrophoresis and ion-exchange chromatography, the electrofocusing method has been used successfully for the separation of isoenzymes [9-10]. It has been established by this method that the electrophoretically homogeneous components A, B, and C of L-amino acid oxidase consist of several enzymatically active proteins differing in their isoelectric points [6].

In the present paper we describe the results of the separation by the isoelectric electrofocusing method in a thin layer of polyacrylamide gel of the electrophoretically homogeneous form III of yeast enolase (II) obtained by the preparative method of disc electrophoresis, and also of a partially purified preparation of enolase.

Electrofocusing in polyacrylamide gel has shown that the electrophoretically homogeneous molecular form III of yeast enolase consists of 10-11 protein components differing in their isoelectric points which lie in the pH range from 4.95 to 7.0 (Fig. 1). Zones 3-4, containing the bulk of the protein, are located in the pH range from 6.4 to 6.9. Enolase activity was also found in this range, and no enzyme activity was found below pH 6.2.

The use of an ampholyte with a pH range of 3-10 under the conditions mentioned does not permit the achievement of a separation ensuring the determination of enzymatic activity in the individual zones present in the range showing enolase activity. For this purpose it is necessary to use an ampholyte with a narrower range, especially pH 6-8, since on electrofocusing with an ampholyte having pH 5-7 the region of interest to us is located under the given conditions in the immediate neighborhood of the electrode (Fig. 2).

A partially purified enzyme preparation containing 5-6 electrophoretically different components was separated into 25-28 individual protein zones located in the pH range from 4.37 to 7.6 (Fig. 3). The bulk of the protein was concentrated in the zones with pH 5.15-7.0.

EXPERIMENTAL

To obtain the polyacrylamide gel we took a solution containing the following components (the final concentration is shown): 6% of acrylamide, 0.16% of N,N'-methylene-bis-acrylamide, 0.06% of N,N,N',N'-tetramethylethylenediamine, 2% of ampholyte, and 0.00084% of riboflavin. Using 12 × 18 cm glass plates

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 98-100, January-February, 1971. Original article submitted November 24, 1970.

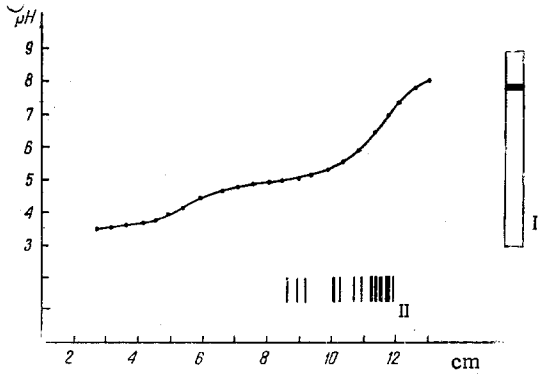

Fig. 1. I) Electrophoregram of the molecular form III of yeast enolase; II) molecular form III separated by electrofocusing; ampholyte pH 3-10.

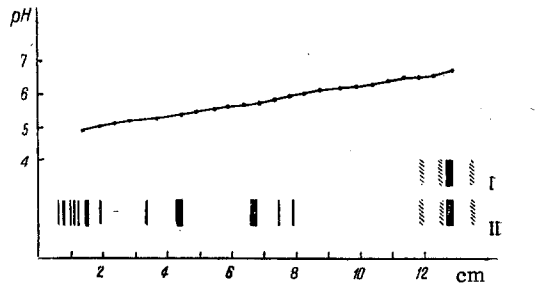

Fig. 2. Molecular form III (I) and partially purified yeast enolase (II) separated by the electrofocusing method; ampholyte pH 5-7.

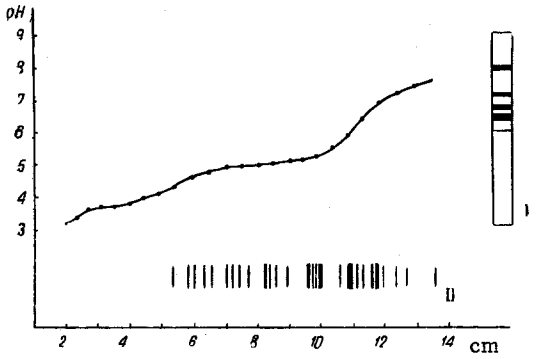

Fig. 3. Electrophoregram of partially purified yeast enolase (I); the same preparation of enolase separated by electrofocusing (II); ampholyte pH 3-10.

(one of them siliconized with dichlorodimethylsilane) arranged at a distance of 1 mm, a thin layer of polyacrylamide gel was obtained. For gel plates with a working area of 10 × 14 cm, 20 ml of the above-described solution was required. After the deaeration of the solution with a water pump, polymerization was carried out at room temperature with illumination for 30 min. The siliconized plate was separated after 2 h. The sample was deposited by means of a piece of chromatographic paper with dimensions of 8 × 9 mm moistened in a desalted solution of the protein under investigation (100-400 μg). The plates with the samples were placed on carbon electrodes previously moistened with solutions of electrolytes: the anode with a 10% solution of H_3PO_4 and the cathode with a 10% solution of ethanolamine. Electrofocusing was carried out at +4°C for 18-20 h. The initial voltage was 110-120 V for 2 h, and this was gradually increased to 300 V. The current strengths in the gel plate with an ampholyte having a pH range of 3-10 were 12 mA at the beginning of the process and 0.8 mA at the end, and with an ampholyte having a pH range of 5-7, 5-6 and 0.4-0.5 mA, respectively. The pH gradient was determined by the elution of pieces of gel 3 × 9 mm cut out with a specially adapted glass tube soon after the completion of the electrofocusing process. Elution was carried out in closed test tubes in 1 ml of double-distilled water for 2 h. The pH values were determined with an LPU-01 laboratory pH-meter equipped with a device for micromeasurements. Staining was performed with a 0.1% solution of Coomassie Blue in ethanol–water–acetic acid (45 : 45 : 10) for 1 h. The unbound dye was washed out with ethanol–water–acetic acid (35 : 60 : 5).

SUMMARY

The heterogeneity of the electrophoretically homogeneous form of yeast enolase has been established by the isoelectric electrofocusing method.

LITERATURE CITED

1. H. Svensson, Acta Chem. Scand., 15, 325 (1961).
2. H. Svensson, Acta Chem. Scand., 16, 456 (1962).
3. O. Vesterberg and H. Svensson, Acta Chem. Scand., 20, 820 (1966).
4. C. Wrigley, Science Tools, 15, 17-23 (1968).
5. G. Dale and A. L. Latner, Lancet, 1, No. 7547, 847-848 (1968).
6. M. B. Hayes and D. Wellner, J. Biol. Chem., 244, No. 24, 6636, 6644.
7. N. Catsimpoolas, Anal. Biochem., 26, No. 3 (1969).
8. Z. L. Awdeh, A. R. Williamson, and B. A. Askonas, Nature, 219, No. 5149, 66-67 (1968).
9. W. Berndt, N. Hennrich, and H. Lang, Z. Anal. Chem., 243, 475-482 (1968).
10. I. E. A. McIntosh, Biochem. J., 114, 463 (1969).
11. M. A. Geidan and R. A. Zhagat, Biokhimiya, 35, No. 5, 1051 (1970).