THE COMPOSITION OF THE RABBIT MUSCLE TRIOSEPHOSPHATE ISOMERASE ISOZYMES OF TWO DIFFERENT SUBUNITS

Wolfgang K.G.KRIETSCH, Peter G.PENTCHEV, Werner MACHLEIDT and Helgard KLINGENBURG
Institut für Physiologische Chemie und Physikalische Biochemie der Universität München, Germany

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1. Introduction

Burton and Waley [1] described the electrophoretical separation of commercial preparations of triosephosphate isomerase (TPI, EC 5.3.1.1) into three enzymatically active components. Similar results were obtained by Kaplan et al. [2] and Scopes [3, 4] with extracts from human erythrocytes and pig and rabbit muscle, respectively. In our studies on isolated TPI from rabbit muscle and liver, we came to the same results [5].

TPI from rabbit skeletal muscle is a dimer and has two subunits with a molecular weight in the range of 25,000–29,000 [5, 6]. Coulson et al. [7] and MacGregor and Waley [8] suggested that TPI is composed of two identical subunits. Burton and Waley [1] and Kaplan et al. [2] discussed three different configurational forms of the same protein.

Now we have isolated and characterized the three forms of rabbit muscle TPI [9]. On the basis of hybridization experiments and peptide profiles it will be demonstrated that the electrophoretical pattern mentioned above is caused by the existence of two similar but non-identical TPI subunits.

2. Methods

TPI was isolated through ammonium sulfate precipitation and crystallization as outlined by Czok and Bücher [10]. The isozymes were separated by chromatography on a DEAE-Cellulose (Serva GmbH, Heidelberg, Germany) column (2 × 100 cm) with a linear gradient from 215 mM triethanolamine buffer pH 7.0 and 215 mM triethanolamine buffer pH 7.0 and 20 mM NaCl [9].

Starch gel electrophoresis was carried out in a vertical gel apparatus suggested by Smithies [11] with a gel composed of 13% starch and 0.5% agarose. The buffers were those used by Kaplan et al. [2]. Following electrophoresis for 26 hr and 13 v/cm at 4° the gel was sliced horizontally and stained with amido black.

For tryptic digestion the isozymes were denatured with 5% trichloroacetic acid and then redissolved in 0.5 M ammonium hydrogen carbonate buffer pH 8.6. The digestion was performed by incubating the protein 3 times for 2 hr at 37° each time with 1% trypsin (Serva GmbH, Heidelberg, Germany) with respect to the protein. In a second set of experiments diphenylcarbamylchloride (DCC)–trypsin was used instead of trypsin (Serva GmbH). The peptides were dried in vacuo over H_2SO_4/NaOH and redissolved in 0.1 M citrate buffer pH 2.75. An insoluble residue of undigested protein or insoluble peptides was centrifuged off. The peptides were analyzed by automatic chromatography on a 0.1 × 50 cm column with the cation exchange Aminex A 6 (Bio-Rad Laboratories, Richmond, California), according to a method developed in our laboratory [12]. The peptides in the effluent are detected by continuous reaction with trinitrobenzene sulfonic acid and flow-cell photometry at 334 nm.

3. Results and discussion

3.1. *Hybridization*

The three purified forms of TPI (α, β and γ; α is the

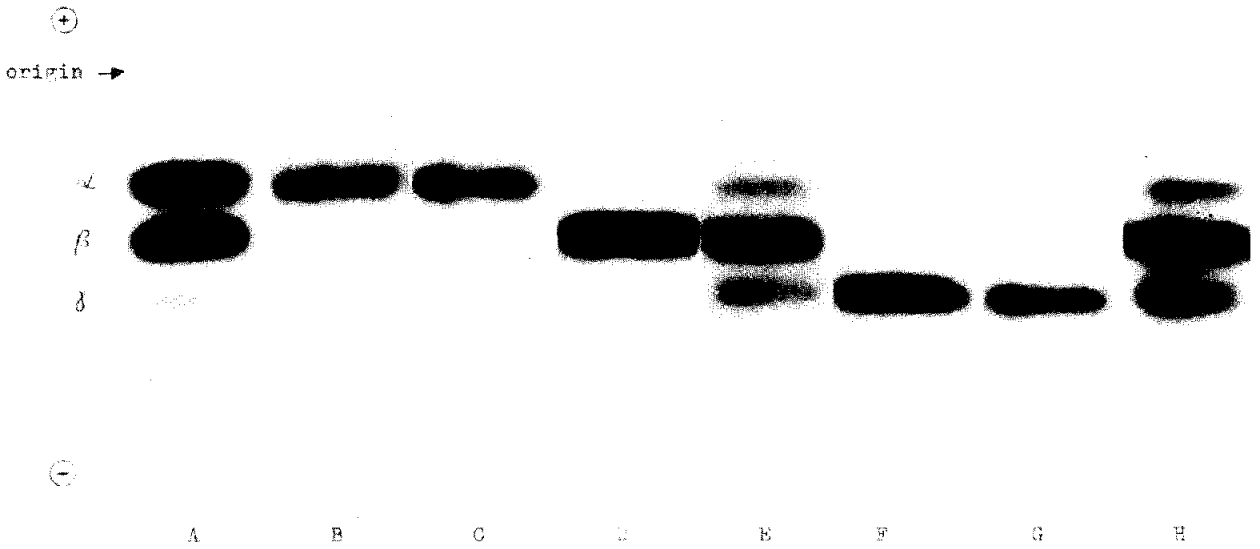

Fig. 1. Starch gel electrophoresis of TPI. (A) native TPI from rabbit muscle, (B) purified α form, (C) α form after hybridization, (D) purified β form, (E) β form after hybridization, (F) purified γ form, (G) γ form after hybridization, (H) hybridization of an α and γ mixture.

slowest anodically migrating form) were dissolved in 2 M NaCl. The hybridization was carried out similar to the method described by Markert [13]. The samples were frozen in liquid nitrogen, stored for 30 min at −30° and then incubated for 30 min at +40°. This procedure was repeated at least 6 times. While the α and γ enzymes remained unchanged in their electrophoretic behaviour by this procedure, from the β enzyme emerged three forms with the same electrophoretic mobility as the original α, β and γ forms (fig. 1). When an α and γ mixture was submitted to hybridization the β isozyme was formed (fig. 1). The amount of the α, β and γ isozymes in native TPI is different from that in hybrids, which show the statistically expected proportions (fig. 1).

These hybridization experiments suggest that the TPI has two different subunits, here coded A and B. The subunit composition of the three isozymes α, β and γ is then AA, AB and BB, respectively. No hybrids were found when a mixture of muscle and yeast TPI [5] was incubated under the same conditions.

3.2. *Tryptic peptides*

According to the amino acid composition [5] one should find about 67 peptides after digestion with trypsin, when the subunits are completely different, and 34 with identical subunits. In the chromatography of the peptides from the digestion with DCC treated trypsin we counted approximately 30 peptides (fig. 2).

The comparison of the peptide chromatograms shows at least three different peptides (fig. 2 and 3) in

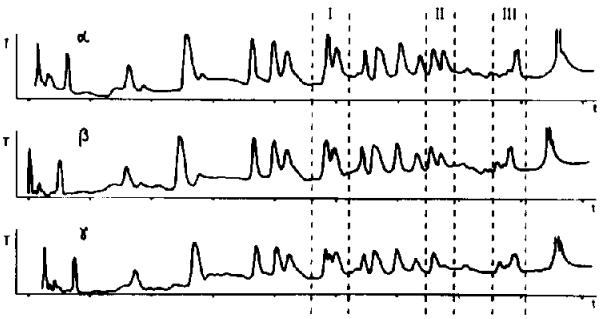

Fig. 2. Column chromatography of tryptic peptides of the three TPI isozymes (α, β and γ). The peptides chromatographed in each run corresponded to approximately 1 mg protein. Experimental conditions are given in Methods. The differing regions I, II and III are shown in detail in fig. 3.

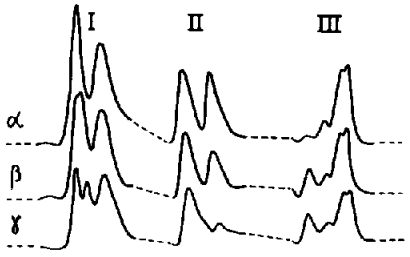

Fig. 3. Detailed reproduction of the differing regions I, II and III from the peptide profile in fig. 2.

the α (AA) and γ (BB) isozymes. In all cases the β (AB) isozyme contains the peptides from the α as well as from the γ isozyme. In a different set of experiments digestion was carried out with trypsin not treated with DCC. As expected, more peptides are found but there are also three differences in the same regions of the peptide patterns from the α and γ isozymes, respectively, and again the β isozyme is intermediate between α and γ peptide profile.

These experiments provide strong evidence that the rabbit muscle TPI isozymes are composed of two different polypeptide chains, coded on two cistrons. This could also be an explanation for the multiple forms of TPI observed in human erythrocytes [2], pig muscle [3] and rabbit liver [5].

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