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Chemical evidence for the presence of subunits in glyceraldehyde-3-phosphate dehydrogenase

The fingerprint of a tryptic hydrolysate of crystalline glyceraldehyde-3-phosphate dehydrogenase, (D-glyceraldehyde-3-phosphate: NAD oxydoreductase (phosphorylating), EC 1.2.1.12), isolated from swine muscle, is shown in Fig. 1. It is to be seen from the tracing that only 29 components (16 of them giving an intense colour reaction with ninhydrin and 13 a fainter one) could be separated under the experimental conditions applied. The basic components appeared to be, in addition to free lysine and arginine, mostly small peptides. Among the acidic components the one marked with "30" corresponds to free glutamic acid. The neutral components did not fractionate well under the conditions of fingerprinting. A considerable amount of material remained at the starting point.

From the tryptic hydrolysate 20% of the dissolved material precipitated at 3.3% end-concentration of trichloroacetic acid. The precipitate contained neutral and acidic components, while the basic peptides remained in the supernatant.

The trichloroacetic acid-insoluble fraction was oxidized with performic acid, then gel-filtrated on a Sephadex G-25 column in order to remove components of small molecular weight. The high-molecular-weight fraction could be separated into at least three chief components by chromatography on cellulose powder (Fig. 2). Fraction A was a peptide, containing 2.2% cysteic acid, and had a minimal molecular weight of about 7700. The analysis of this peptide is in progress.

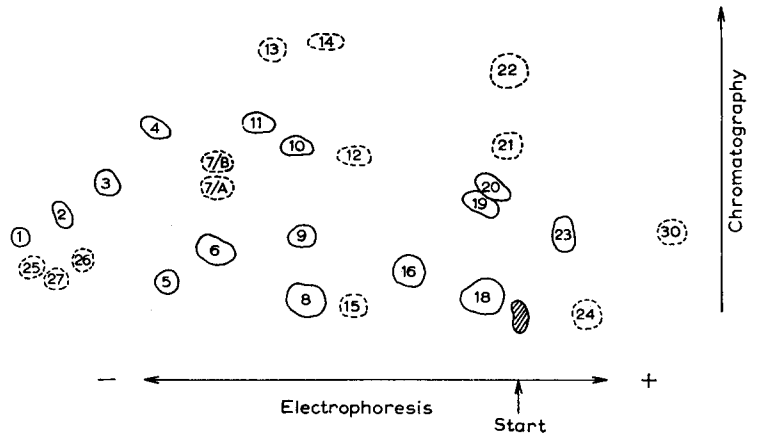

Fig. 1. Tracing of a typical fingerprint of tryptic digest. Digestion: The crystalline trypsin (EC 3.4.4.4) (Worthington) was preincubated in 0.04 N HCl at 37° for 16 h. The hydrolysis of a 1% suspension of heat-denatured, acetone-dried glyceraldehyde-3-phosphate dehydrogenase sample was carried out at pH 7.9 in an autotitrator with 1/30 weight of trypsin up to 87% digestion. Paper electrophoresis: a horizontal electrophoresis apparatus was used with a coolingplate of 40 × 40 cm, constructed in our laboratory. 30 V/cm, pH 5, 0.2 h. Chromatography: ascending, *iso*-amylalcohol–pyridine–water (35:35:30) (ref. 12).

The molecular weight of glyceraldehyde-3-phosphate dehydrogenase is 120 000–140 000 based upon data in literature[1–5]. In our experiments we calculated on the basis of a molecular weight of 140 000. According to the amino acid analysis of BENEY[6], swine-muscle glyceraldehyde-3-phosphate dehydrogenase, contains 105 and 42 residues of lysine and arginine, respectively. Accordingly the tryptic hydrolysate was expected to contain at least 140–150 peptides. The fingerprint shows, however,

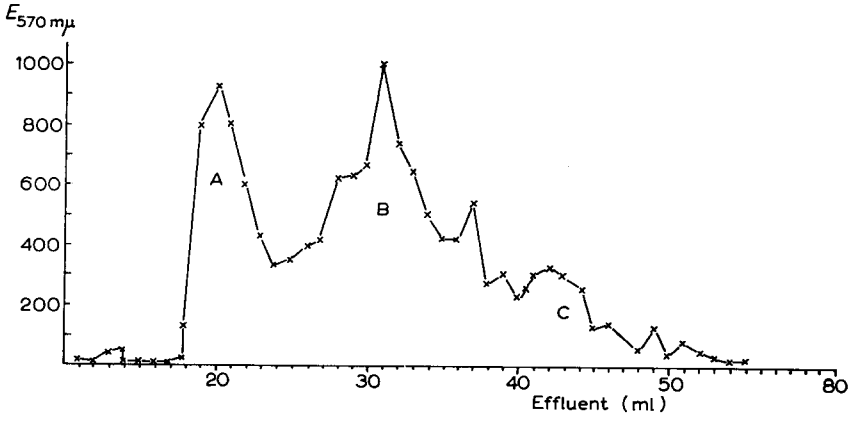

Fig. 2. Chromatography of the trichloroacetic acid-insoluble fraction. 50 mg of the trichloroacetic acid-insoluble fraction was dissolved in 0.1 ml 1% ammonium hydroxide, and diluted with 0.2 ml pyridine. The solution was transferred to a Whatman standard grade cellulose-powder column 1 × 20 cm previously equilibrated with the pyridine–*iso*-amylalcohol–water mixture[12]. Elution was allowed to take place at a rate of 3 ml/h; the effluent was collected in fractions of 1 ml. 0.1 ml of each fraction was hydrolysed with alkali, and the ninhydrine colour density was read, according to the method of HIRS, MOORE AND STEIN[13].

that only some of these could be detected on tryptic hydrolysis. Taking into account the free lysine and arginine and the core fraction as well—which contained some of these basic amino acids—it had to be assumed that the enzyme is built up from several similar or identical subunits.

In special cases the number of terminal groups may give an indication of the higher structure of proteins. According to the analyses of VELICK[7], the glyceraldehyde-3-phosphate dehydrogenase isolated from rabbit skeletal muscle contains two terminal valines. In contrast to our earlier data[8], we have recently found that native glyceraldehyde-3-phosphate dehydrogenase, isolated from swine muscle, contains 2.4–2.7 N-terminal valine residues calculated on the basic of a molecular weight of 140 000. To detect the N-terminal valines we used the procedure of LEVY[9], but with a longer reaction time. The existence of three subunits of glyceraldehyde-3-phosphate dehydrogenase seems therefore probable.

These results are in good agreement with the strong binding of 3 moles of NAD by the enzyme[10] and with the finding of DVORNIKOVA[11], who claims that the molecular weight of glyceraldehyde-3-phosphate dehydrogenase is only 42 000 at the beginning of crystallization and that the value of 140 000 can be measured only after the enzyme solution has stood for a prolonged time.

On the basis of our experiments, we propose that the glyceraldehyde-3-phosphate dehydrogenase is built up from three identical subunits of molecular weight about 47 000. Our results are in good agreement with the conclusions of HARRIS and coworkers[14], according to whom the enzymatic hydrolysate of glyceraldehyde-3-phosphate dehydrogenase isolated from rabbit muscle contains three identical peptides, each bearing the functionally active SH group, corresponding to the polymer structure of the protein.

The experiments will be published in details in Acta Physiologica Hungaricae.

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