PEPTIDE MAPPING BY LIMITED PROTEOLYSIS OF FOUR PYRUVATE KINASE ISOZYMES

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1. Introduction

There are several multiple molecular forms of pyruvate kinase (ATP: pyruvate 2-*O*-phosphotransferase, EC 2.7.1.40) in various animal tissues. The three main isozymes, i.e., type L, type M_1 and type M_2, have been highly purified and their molecular natures have been investigated. Type L differs from type M_1 or type M_2 in immunological properties [1,2] and amino acid composition [3,4], and thus considered to be a product of separate gene. On the other hand, type M_1 and type M_2 have very similar in immunological properties [5] and amino acid compositions [3,4], and thus it still uncertain whether they are different gene products [6,7] or not [8].

Another type of isozyme, type R, is present in erythrocytes. Type R is closely similar to type L in immunological properties [2], genetic properties [9], and amino acid composition [4]. However, various models have been proposed to explain its anomalous electrophoretic mobility [9,10], although there is no general agreement on this problem.

For confirmation of the structural differences of these isozymes, their primary structures must be compared. The present work demonstrates the similarities and the dissimilarities in the peptide maps obtained by limited proteolytic digestion of the four pyruvate kinase isozymes.

The peptide patterns of type M_1 and type M_2 were quite different, and so the two isozymes were concluded to be products of separate genes. The peptide patterns of type L and type R were very similar although type R had a few additional components. Thus, it is concluded that type R and type L share a common structure, but that type R may possess a minor additional structure.

2. Materials and methods

Four pyruvate kinase isozymes were completely purified from rat tissues as in [4]. Type M_1 was from skeletal muscle, type M_2 from AH-130 Yoshida ascites hepatoma cells, type L from liver and type R from erythrocytes. The enzymes were denatured in the presence of 6 M guanidine hydrochloride and dithiothreitol, and the cysteine residues were carboxymethylate [11]. The preparations were then analyzed as in [12]. They were dissolved in 0.1% SDS solution, partially digested with *Staphylococcus aureus* V 8 protease (Miles Labs) or papain (Sigma) and the resulting products were analyzed by electrophoresis.

Electrophoresis was performed in slab gel, containing a linear gradient of 12–20% acrylamide, with the discontinuous Laemmli system [13]. The approximate subunit molecular weights were estimated. For this, the standard proteins used with their molecular weights were: RNA polymerase β', 165 000; RNA polymerase β, 155 000; bovine albumin, 68 000; bovine liver catalase, 57 500; RNA polymerase α, 39 000; rabbit muscle lactate dehydrogenase, 35 000; trypsin inhibitor, 21 500; bovine hemoglobin, 16 000; cytochrome *c*, 12 400.

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3. Results and discussion

Figure 1 shows the electrophoretic patterns of the carboxymethylated samples. Type M_2 contained homogeneous subunits (mol. wt 61 000). Type R contained major large subunits (mol. wt 62 000) and minor small subunits (mol. wt 57 000) as in [4,10]. Laemmli's electrophoretic method revealed the microheterogeneities of type M_1 and type L, which were not detected by the method in [14]. Type M_1 contained very closely adjacent bands (mol. wt 59 000, 57 000). Type L contained major small subunits (mol. wt 57 000) and minor large subunits (mol. wt 60 000) and the minor small subunits of type R were the same size as the major small subunits of type L. These micro-heterogeneities may be artifacts formed during purification or they may be natural phenomena associated with the in vivo proteolytic conversion of the enzymes [10].

The four carboxymethylated samples were partially digested with *St. aureus* V 8 protease or papain, and the resulting products were analyzed by electrophoresis (fig.2,3). The patterns of peptide fragments of type M_1 and type M_2, produced with either V 8 protease or papain had only a few bands in common. On the other hand, those of type L and type R were very similar, but type R had a few additional bands. These results indicate that the primary structures of type M_1 and type M_2 are fundamentally different, and so the two isozymes must be products of separate genes. Type R seems to have a large portion of its primary structure in common with type L and to possess some additional structure. The larger subunit size of type R can also be explained by this additional structure.

Native human type R was reported [15] to be converted to a form closely similar to type L by mild tryptic digestion and an analogous conversion might occur in erythrocytes [15]. These findings and the present data suggest the occurrence of proteolytic modifications of pyruvate kinase isozymes, but further studies are required on their physiological meaning.

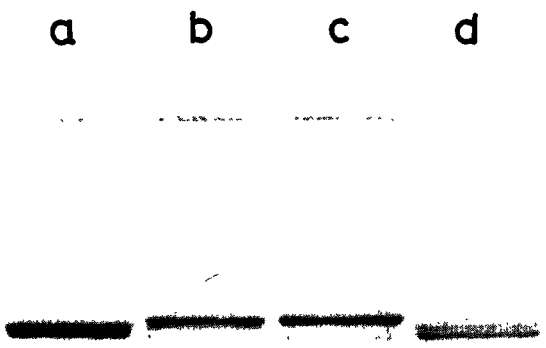

Fig.1. SDS–polyacrylamide gel electrophoresis of the purified carboxymethylated samples of the four pyruvate kinase isozymes. About 1 μg of each isozyme was applied. (a) type M_1; (b) type M_2; (c) type R; (d) type L.

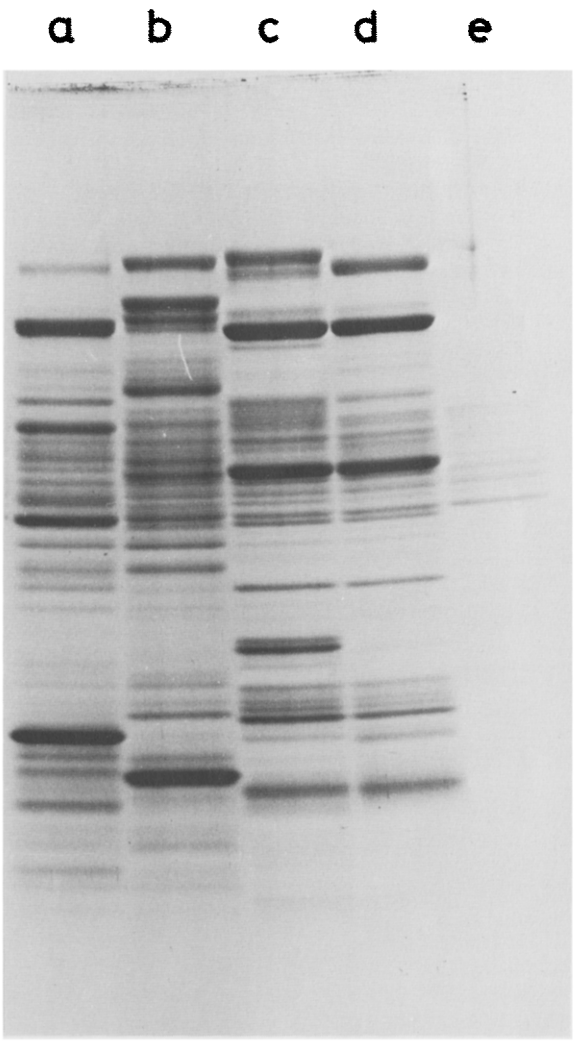

Fig.2.

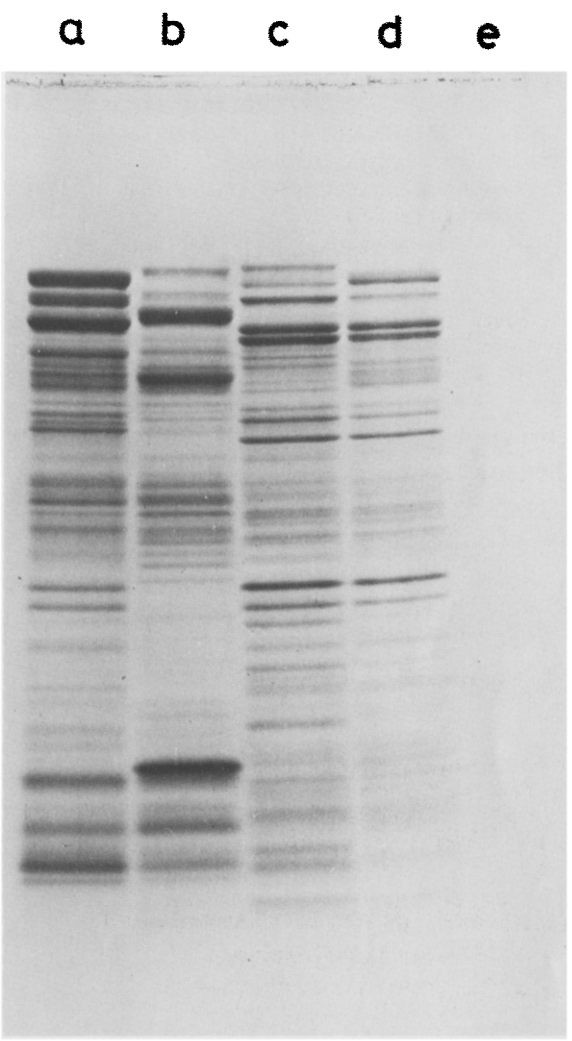

Fig.3.

Fig.2,3. Electrophoretic patterns of partial digestion products of carboxymethylated samples after digestion with *St. aureus* V 8 protease (fig.2) or papain (fig.3). Digestion by proteases was carried out as in [12]. V 8 protease was dissolved at ~0.26 mg/ml in the sample buffer and papain, at ~2.6 μg/ml in the sample buffer supplemented with 1 mM EDTA and 5 mM KCN. The carboxymethylated samples were dissolved at 1 mg/ml in the sample buffer. Digestion was carried out by incubating the sample solutions with an equal volume of protease solution for 90 min at 37°C (V 8 protease) or at 63°C (papain). (a) type M_1; (b) type M_2; (c) type R; (d) type L; (e) protease. As shown in fig.2e, the V 8 protease was contaminated with a considerable amount of protein.

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