# THE SUBUNIT STRUCTURE OF HUMAN MUSCLE AND HUMAN ERYTHROCYTE PYRUVATE KINASE ISOZYMES

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## 1. Introduction

In human tissues the pyruvate kinase reaction (ATP: pyruvate phosphotransferase; E C 2.7.1.40) is catalysed by at least three isozymes [1–3] which can be distinguised by their kinetic, electrophoretic and immunological properties: $M_1$ present in muscle, L the major form in liver and $M_2$ found in liver, kidney and adipose tissue. The isozyme present in red cells (R) is kinetically similar to the L isozyme but can be distinguished from it by electrophoresis [2–4]. The R isozyme can be converted into a form which is electrophoretically identical to the L isozyme either by treating with a liver homogenate or repeated freezing and thawing [4]. The molecular structures and interrelationships of the isozymes are unknown. We have examined the human $M_1$ and R isozymes by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and two dimensional fingerprinting of their cyanogen bromide peptides. The evidence is consistent with identical subunits in the $M_1$ tetramer and two pairs of non-identical subunits in the R tetramer. One of the R subunits may be homologous with the $M_1$ subunit but is probably not identical to it.

## 2. Materials and methods

Human erythrocyte pyruvate kinase was prepared as previously described [5]. The methionine content has been reported to be 46 residues per molecule [5]. Human muscle pyruvate kinase was purified by a modification of a published procedure [6].

Cyanogen bromide was purchased from the Aldrich Chemical Co. and iodoacetamide from the Sigma Chemical Co.

Polyacrylamide disc gel electrophoresis at pH 8.7 was by the method of Davis [7] using Buchler apparatus. SDS polyacrylamide disc gel electrophoresis was performed as described by Weber and Osborn [8] using the normal amount of cross-linker. Subunit molecular weights were estimated as described by these authors [8] using bovine serum albumin, rabbit muscle

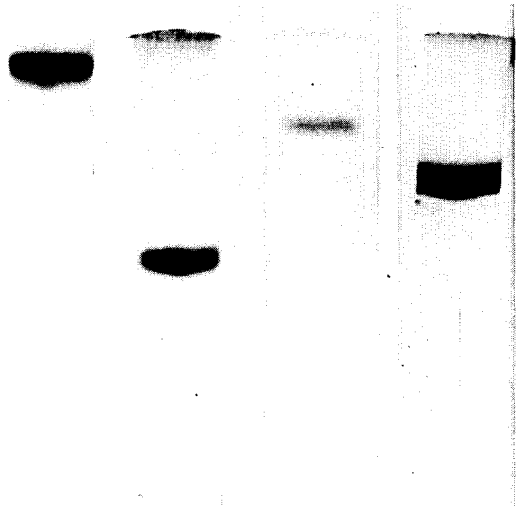

Fig. 1. Polyacrylamide gel electrophoresis of the human muscle and erythrocyte pyruvate kinase isozymes. From left to right the gels contained: 1 and 2 polyacrylamide and SDS polyacrylamide gels of the human muscle isozyme, 3 and 4 polyacrylamide and SDS polyacrylamide gels of the human erythrocyte isozyme. The gels were run at different times and no significance is attached to mobility differences between gels.

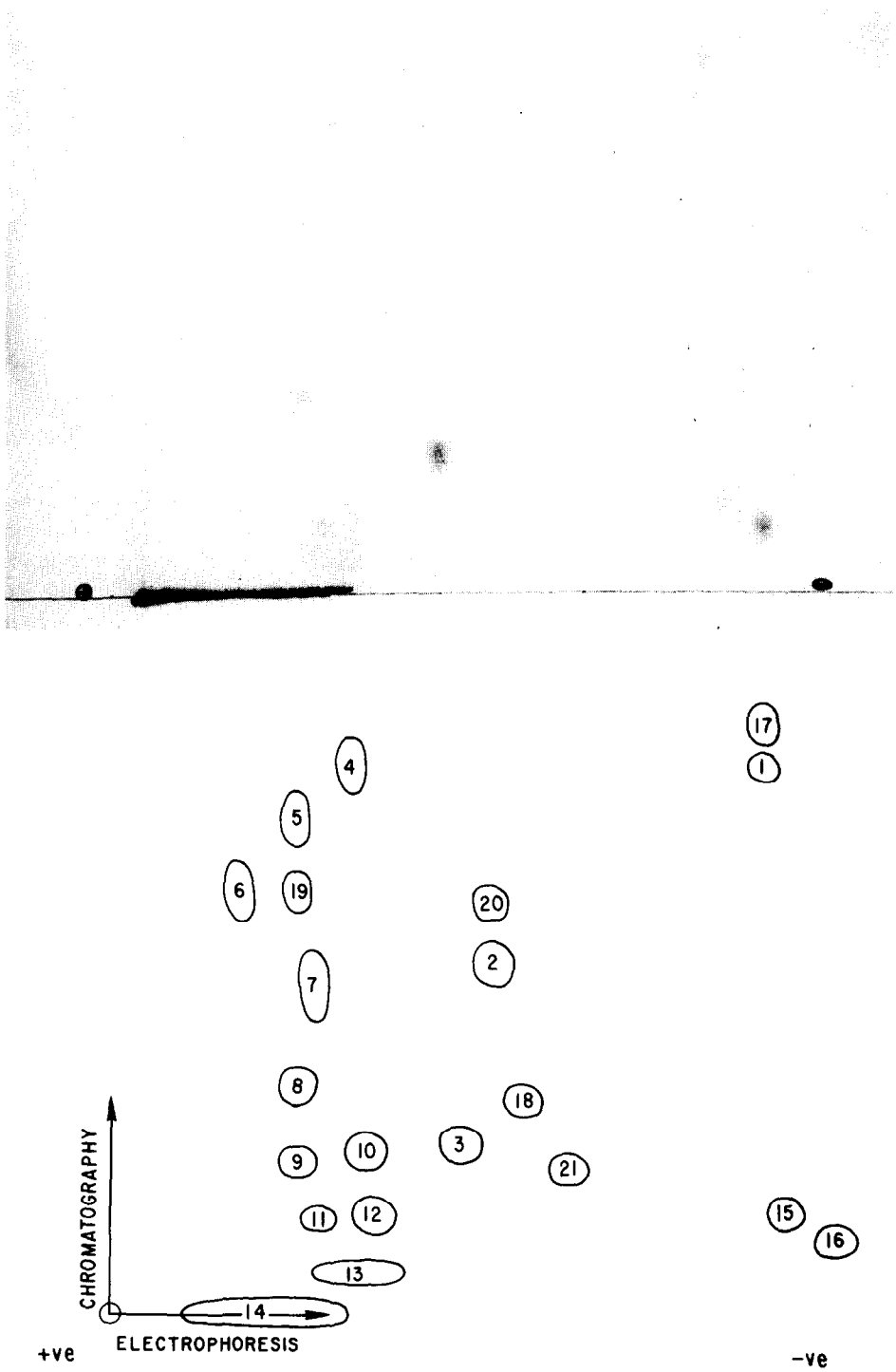


Fig. 2. Fingerprint of the cyanogen bromide peptides of reduced alkylated human muscle pyruvate kinase. Separation conditions are given in the text.

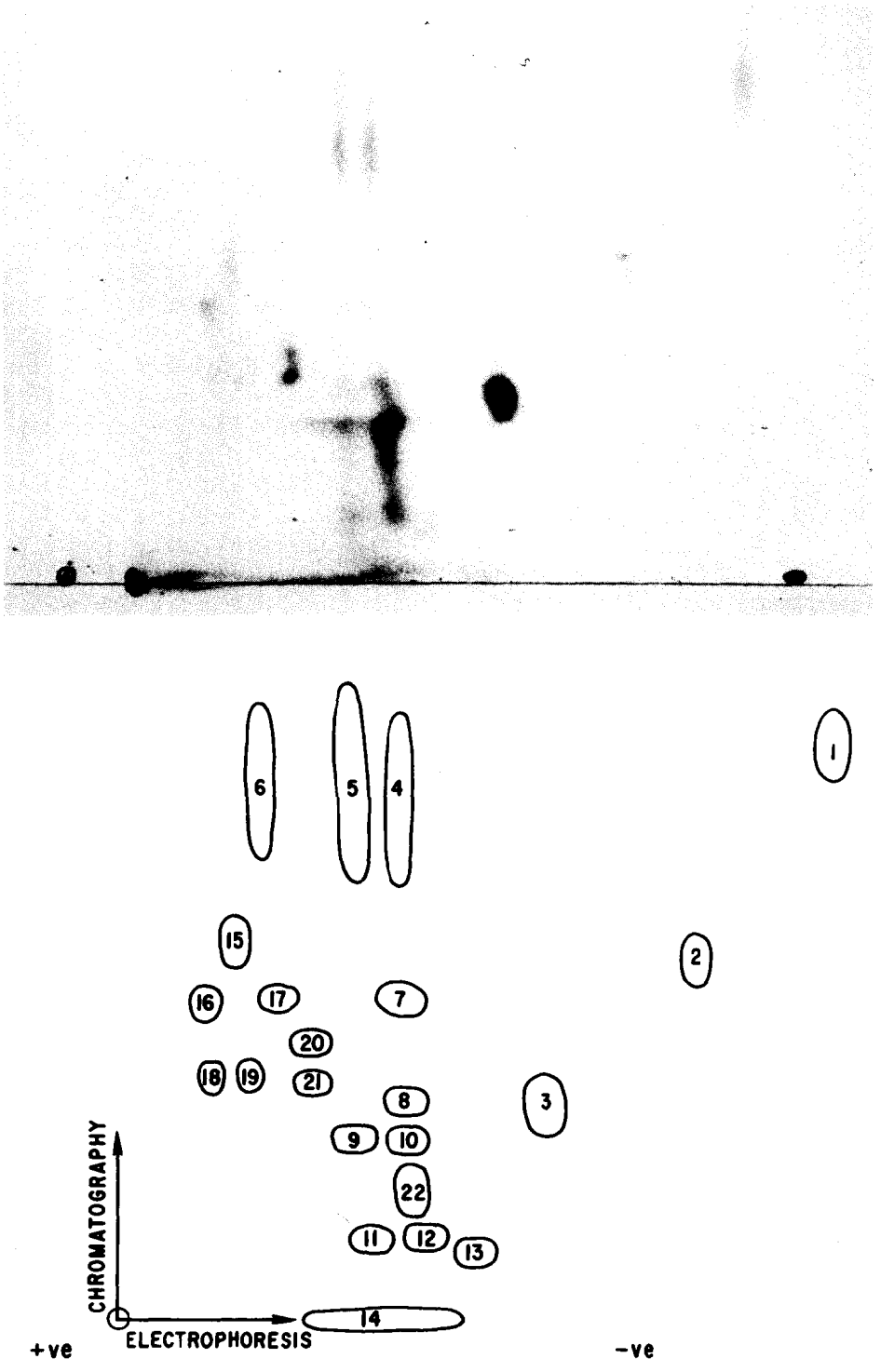


Fig. 3. Fingerprint of the cyanogen bromide peptides of reduced alkylated human erythrocyte pyruvate kinase.

pyruvate kinase, rabbit muscle lactic dehydrogenase and horse heart myoglobin as molecular weight standards.

Reduction and alkylation of cystine residues was carried out by the method of Crestfield, Moore and Stein [9]. The reduced alkylated protein was isolated by acid–acetone precipitation [10].

Cyanogen bromide cleavage was effected in 70% formic acid with a 60-fold molar excess of cyanogen bromide over the calculated methionine content at room temperature for 24 hr [11]. The reaction mixture was then diluted and lyophilized.

Fingerprinting involved overnight descending chromatography on Whatman 3 MM paper in *n*-butanol/acetic acid/water (100/30/75 by vol) in the first dimension and electrophoresis in pH 3.7 pyridine/acetic acid/water (1/10/289 by vol) in the second dimension for 30 min at 3000 V. Peptides were visualized by dipping in 0.5% ninhydrin in acetone.

## 3. Results and discussion

Polyacrylamide gel electrophoresis of the purified $M_1$ isozyme is shown in fig 1. A single peak was also obtained in an ultracentrifuge sedimentation velocity experiment. Amino acid analysis gave a methionine content of 78 residues per molecule.

The $M_1$ isozyme also gave a single band on SDS gel electrophoresis (fig. 1). The electrophoretic mobility [8] corresponded to a subunit mol. wt of 63 000 indicating that the 241 000 dalton human $M_1$ isozyme is a tetramer.

The fingerprint of the cyanogen bromide peptides of the $M_1$ isozyme is shown in fig. 2a with all discrete peptides identified in fig. 2b. A total of 21 peptides have been located. From the 78 residue methionine content of the tetramer, 21 peptides would be expected if the subunits are identical, 41 peptides if two types of subunit are present and 82 peptides if there are four non-identical subunits. We conclude that the human $M_1$ isozyme is a tetramer containing four identical subunits.

As previously reported [5] the purified R isozyme gave a single band on polyacrylamide gel electrophoresis. SDS gel electrophoresis (fig. 1), however, gave two distinct bands with similar mobilities which correspond to mol. wt of approx. 60 000. This suggests that the 225 000 dalton R isozyme [5] is a tetramer containing at least two types of subunit. The fingerprint of the cyanogen bromide peptides of the R isozyme is shown in fig. 3a with the interpretation in fig 3b. Twenty-two cyanogen bromide peptides have been separated. The human R isozyme contains 46 methionine residues per molecule and identical subunits should yield 13 cyanogen bromide peptides, two types of subunit 25 peptides and four non-identical subunits 50 peptides. The close agreement between the 22 peptides observed and the 25 expected for two types of subunit supports the evidence from SDS polyacrylamide gel electrophoresis that the human R isozyme is a tetramer containing two pairs of subunits.

There are considerable similarities in the peptide distributions for the $M_1$ and R isozymes as indicated in fig. 2b and 3b. The peptides numbered from 1 through 14 have similar locations in the two illustrations. This would be expected if the $M_1$ an R subunits are homologous in sequence. If the $M_1$ subunit was identical to one of the R subunits, all of the peptides present in fig. 2 should appear in fig. 3. The peptides identified by the numbers 15 through 20 in fig. 2b do not have positional counterparts in fig. 3 and, for this reason, it is unlikely that either R subunit is identical to the $M_1$ subunit. Further studies on the L and $M_2$ isozymes may clarify the origin of the R subunits. It has been propesed that the R isozyme is a hybrid of the L and $M_2$ isozymes [2,3,12].

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