Muscle and liver pyruvate kinases are closely related: amino acid sequence comparisons

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Received 2 April 1984

Previous evidence has shown that the M_1 and L pyruvate kinase isozymes differ markedly in kinetic and immunological properties, amino acid compositions and peptide maps. However, the amino acid sequence results we present here for the N-terminal region and for a region of the C domain show that the M_1 and L isozymes are very similar. The variable length of the N-terminal sequences also explains the difference in regulation by phosphorylation between the M_1 and L isozymes. The M_1 isozyme lacks the serine residue that has been shown to be phosphorylated in the L isozyme.

Pyruvate kinase *M_1 isozyme* *L isozyme* *Phosphorylation* *Amino acid sequence*

1. INTRODUCTION

Pyruvate kinase (PK, EC 2.7.1.40) is active in all cells during glycolysis, and catalyses the formation of ATP by the transfer of a phospho group from phospho*enol*pyruvate to ADP. There is a requirement for divalent and monovalent cations (usually Mg^{2+} and K^+). Four different isozymes of PK exist in vertebrate tissues: M_1 isozyme in skeletal muscle, M_2 isozyme in kidneys, L isozyme in liver, and R isozyme in erythrocytes. All are tetramers with identical subunits of approximate M_r 60000. These isozymes differ in their kinetic properties, with the M_1 enzyme showing hyperbolic Michaelis-Menten kinetics, and the L enzyme being allosterically regulated (review [1]). The activity of the L isozyme is decreased by phosphorylation whereas the M_1 enzyme is not phosphorylated [2,3].

The control of expression of the 4 PK isozymes is particularly interesting. There is one gene that codes for the L and R isozymes, but there are two different mRNAs [4]. Similarly, it is likely that there is one gene for M_1 PK and M_2 PK [5], but two different mRNAs [6]. Thus, the L and R isozymes are closely related to each other as are the M_1 and M_2 isozymes.

Evidence has shown that the M_1 and L isozymes differ markedly in kinetic and immunological properties, amino acid compositions and peptide maps [1,7]. However, we report here amino acid sequence information that shows that the M_1 and L isozymes are very similar.

2. MATERIALS AND METHODS

2.1. *Purification of M_1 and L pyruvate kinases*

Pyruvate kinase was purified from cat and from rat skeletal muscle by a procedure based on that devised in [8]. The method involves ammonium sulphate precipitation, affinity elution from CM–cellulose by phospho*enol*pyruvate, and gel filtration. The L isozyme was purified from rat liver according to a modification of the method in [9]. In the modified procedure the final chromatography steps on DEAE–cellulose and CM–cellulose were replaced by selective absorption of L PK to Sepharose procion blue MX-R in

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00145793/84/$3.00

the presence of Mg^{2+}, and elution by removal of the divalent cation (in preparation).

2.2. *Isolation of peptides*

Reduced and carboxymethylated cat M_1 and rat L isozymes were treated with CNBr (100-fold molar excess over methionine residues for 24 h at room temperature), and the resulting peptides were separated on C_{18} and phenyl reverse-phase columns by HPLC. Peptides CN24, CN40, CN34a, CB1, CN34, CN9, CN1 and CB2 were purified in this way. Peptide CN24SP2 was purified by HPLC from a staphylococcal protease digest of CN24 (that had a blocked N-terminus). The cat M_1 isozyme was also digested with clostripain [10]. The protein was reduced, carboxymethylated and citraconylated prior to digestion, and the protease was pretreated with 10 mM DTE for 2 h at 4°C. Digestion was done at pH 7.5 for 2 h at 37°C at a protein/protease ratio of 125:1 and at 1.5 mg protein/ml of 20 mM ammonium bicarbonate. The peptides were isolated by prefractionation on Sephadex followed by Aminex A5 or SP-Sephadex cation exchangers, as well as by HPLC on C_{18} or phenyl reverse-phase columns.

2.3. *Sequence determination*

Automated liquid-phase sequencing of the CNBr peptides and CL13 was done with a Beckman 890C sequencer fitted with the Beckman cold-trap accessory. The details of the sequencer operation and the identification of the phenylthiohydantoin derivatives have been described [11]. Automated solid-phase sequencing of the clostripain peptides was done as in [12], except that the amino acid phenylthiohydantoins were identified by HPLC.

3. RESULTS AND DISCUSSION

The amino acid sequences of two regions of L and M_1 PK are compared in fig.1. The N-terminus of rat M_1 PK is unblocked (although cat M_1 PK is blocked), and this allowed the N-terminal sequence of 35 residues to be established directly. The cat M_1 PK sequence was obtained from sequence analysis of clostripain and CNBr peptides as shown in the figure. The initial and repetitive yields of representative peptides are given in table 1. The two M_1 PK sequences are identical except for the N-terminal 7 residues where there are 4 amino acid replacements (89% sequence identity over the 35 residues compared).

The M_1 and L PK sequences are clearly closely related, with 66 of the 104 (or 63%) of the residues compared being identical. It is apparent, however, that the N-terminal portions of the two isozymes are quite different. The sequence determined in [13] corresponded to a CNBr peptide derived from rat L PK, and it is therefore possible that there may be additional residues N-terminal to the peptide. If a comparison is made of residues 8–63 then 77% of the residues are identical; 56% of the residues from 407–447 are identical.

The high resolution crystallographic structure of cat M_1 PK [14] shows that each subunit consists of 3 distinct domains. The active site is located between domains A and B at the carboxy end of the eight-stranded β-barrel that forms the core of the largest domain, A. The N-terminal, approximately 10 residues appear to form a relatively flexible portion of the subunit before the polypeptide chain continues into a more ordered region and then into the first β-strand of domain A. A comparison of the sequences of cat M_1 PK, chicken muscle PK and yeast PK shows that domain A is more highly conserved than either domain B or C, and that there are substantial variations at the N-terminus (the yeast enzyme is 22 residues shorter at the N-terminus) [15]. The sequence results reported here show that L and M_1 PK also have similar structural relationships: (i) the two isozymes differ in length at the N-terminus; (ii) residues 11–64 from domain A show less variation than residues 407–447 from domain C.

The variable N-terminal sequences also provide a simple structural basis to explain the difference in regulation by phosphorylation between M_1 and L PK. This explanation has already been suggested from a comparison of the sequence of chicken muscle PK deduced from mRNA [16] with the sequence of phosphoserine-containing peptides from rat and pig L PK reported in [13]. Our results comparing the N-terminal sequences of the M_1 and L isozymes from the same mammalian species confirm this explanation. It is apparent by homology that the C-terminal portion of the phosphopeptide from L PK corresponds to the N-terminal part of

M_1 PK. The muscle enzyme is shorter at the N-terminus by at least 12 residues, and thus lacks the serine flanked by positive charges that is the characteristic site for specific phosphorylation and dephosphorylation in L PK and similarly modulated enzymes.

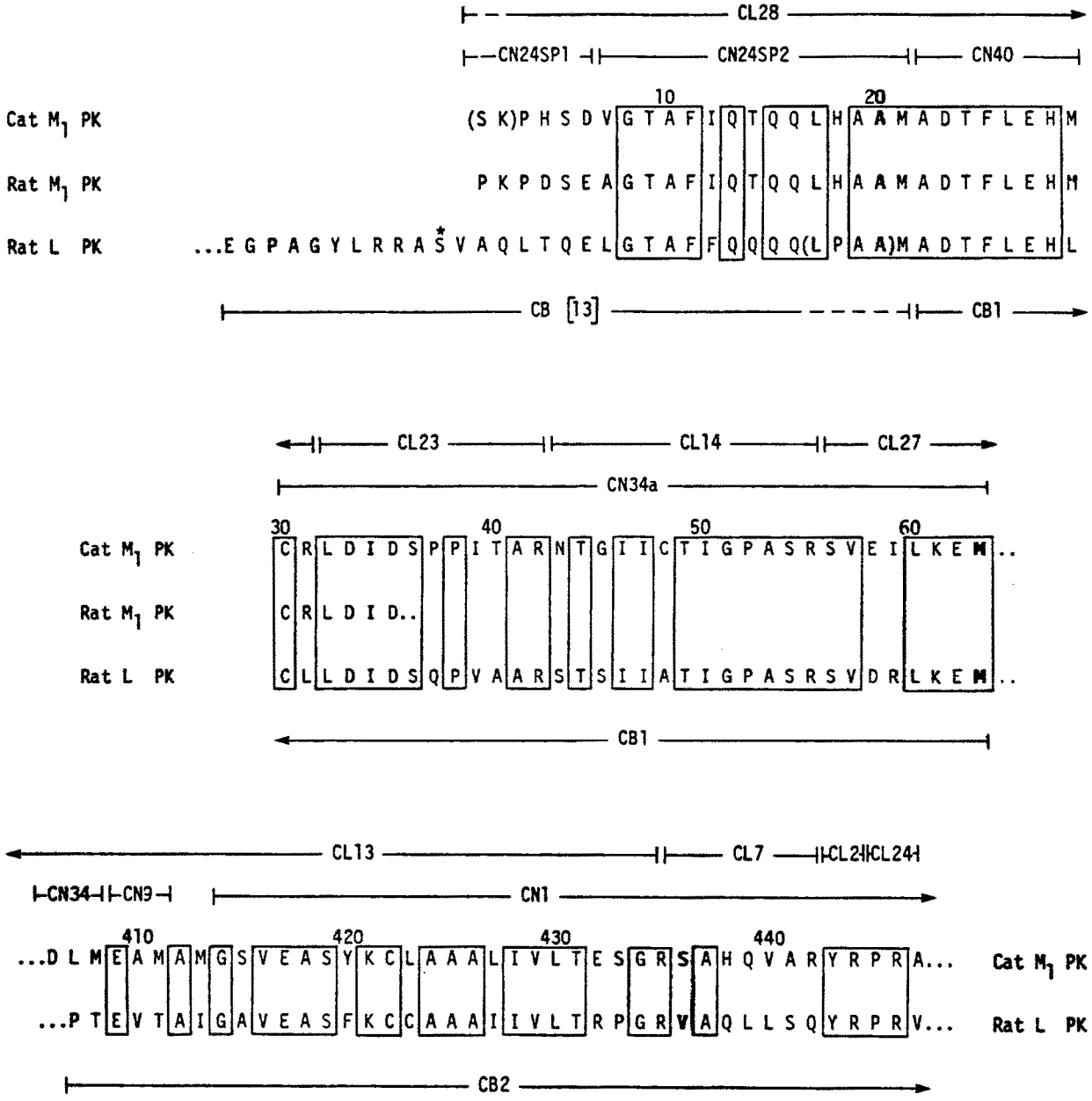

Fig.1. Comparison of amino acid sequences from M_1 and L pyruvate kinases. Peptides designated CN were derived from CNBr cleavage of cat M_1 PK, CL from clostripain digestion of cat M_1 PK and CB from CNBr cleavage of rat L PK. The solid lines indicate that the sequence has been determined; the dashed lines show any unsequenced regions. The residues are numbered according to the cat M_1 PK sequence. Identical residues are boxed in. The serine that can be phosphorylated in L PK is shown by an asterisk.

Table 1

Initial and repetitive yields of sequenced peptides

	Initial yield		Repetitive yield (%)
	nmol	%	
Rat M_1 PK	8	ND	95
CN24SP2	9	45	91
CN40	23	45	93
CN34a	12	52	95
CB1	8	40	97
CL13	10	36	90
CN1	3	35	92
CB2	6	30	95

The repetitive yields were calculated by linear regression analysis; recoveries of PTH-Ser, PTH-Thr, PTH-Ile, PTH-CMCys and PTH-Trp were not included in the analysis. ND, not determined

ACKNOWLEDGEMENTS

We are grateful to Dr Hilary Muirhead for X-ray crystallographic information. The work was supported by the Science and Engineering Research Council, the Medical Research Council and the Deutsche Forschungsgemeinschaft (Schi 145/6-1.2). We also wish to thank Ms Birgitt Sorg for her excellent technical assistance with the clostripain peptides.

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