Complete nucleotide and deduced amino acid sequences of rat L-type pyruvate kinase

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Received 6 November 1985

Four overlapping cDNA clones for L-type pyruvate kinase (PK-L) were isolated from carbohydrate-induced rat liver cDNA libraries. They contained all the coding sequence of the enzyme from the 7th codon and the entire 3'-untranslated extension up to the poly(A) tail. The sequence of the first 7 codons and that of the 5'-untranslated region were determined by primer extension. The analyzed PK-L mRNA has 19 5'-untranslated bases, 1629 coding bases and 1281 3'-untranslated bases without the poly(A) tail; it corresponds to the heavier, 3.2 kb species of the L-type mRNAs. The codons for the phosphorylatable site are located at the 5'-end of the messenger. The unusually long 3'-untranslated extension contains a repetitive element complementary to the 'brain-specific' identifier sequence described by Sutcliffe et al. [(1982) Proc. Natl. Acad. Sci. USA 79, 4942–4946].

Pyruvate kinase *Amino acid sequence* *cDNA* *Nucleotide sequence* *Phosphorylatable site*

1. INTRODUCTION

L-type pyruvate kinase (PK-L) is a liver-specific enzyme which is thought to play a major role in regulation of glycolysis. It is accurately regulated by diet and hormones at a pretranslational level [2] and, post-translationally, by phosphorylation-dephosphorylation [3,4]. Except for the C-terminal end [5] and for the phosphorylated peptide [6,7], the amino acid sequence of the enzyme is unknown.

The liver L enzyme seems to be encoded by the same gene as the erythroid cell-specific L' pyruvate kinase [8,9]. Structure comparison between L and L' subunits indicated that they were identical [10,11] except at the level of the phosphorylated peptide which was heavier in L' than in L subunits [11]. The phosphorylated serine was shown to be located near to an extremity of the L and L' subunits [11]. Simon et al. [11] concluded therefore that L and L' subunits differed by the extremity that carries the phosphorylatable site.

cDNA clones for rat PK-L were recently isolated by Simon et al. [12] and by Noguchi et al. [13].

Here we report the complete nucleotide sequence of the PK-L mRNA and the deduced amino acid sequence. The phosphorylatable serine appears to correspond to the 12th codon and is therefore located at the 5'-end of the L subunit. The 3'-untranslated sequence is unusually long and contains a repetitive element which is complementary to the 'brain-specific' identifier sequence described by Sutcliffe et al. [1].

2. MATERIALS AND METHODS

Clones (11 C6, 12 H2 and 2 B8), complementary to the 3'-part of the messenger, have been isolated from the library described by Simon et al. [12]. They have been subcloned in the single-stranded M-13 mp 10 and mp 11 bacteriophages and sequenced by Sanger's dideoxy chain termination method [14].

Clone PK G4 (fig.1) was isolated from a second carbohydrate-induced rat liver cDNA library constructed from non-fractionated poly(A)-containing

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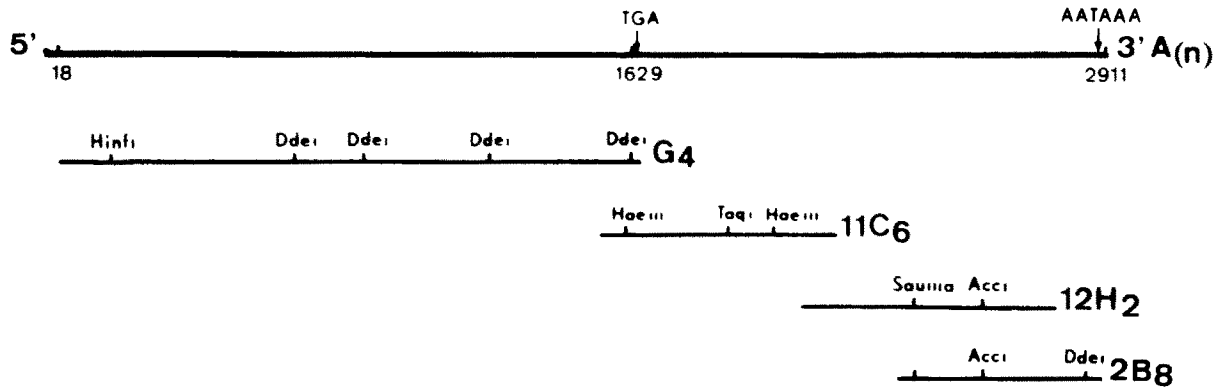


Fig.1. Restriction map analysis of PK-L cDNA clones. The upper part of the figure represents all the length of PK-L mRNA without the poly(A) tail. Position +1 designates the A nucleotide of the AUG initiator codon.

RNAs (Simon et al., unpublished). About 20×10^3 recombinant clones were screened by hybridization with the 11 C6 insert. 18 positive clones were detected. The PK G4 clone was selected for further investigations because of its length (1.8 kb) and the absence of hybridization with the extreme 3'-probes (12 H2 and 2 B8).

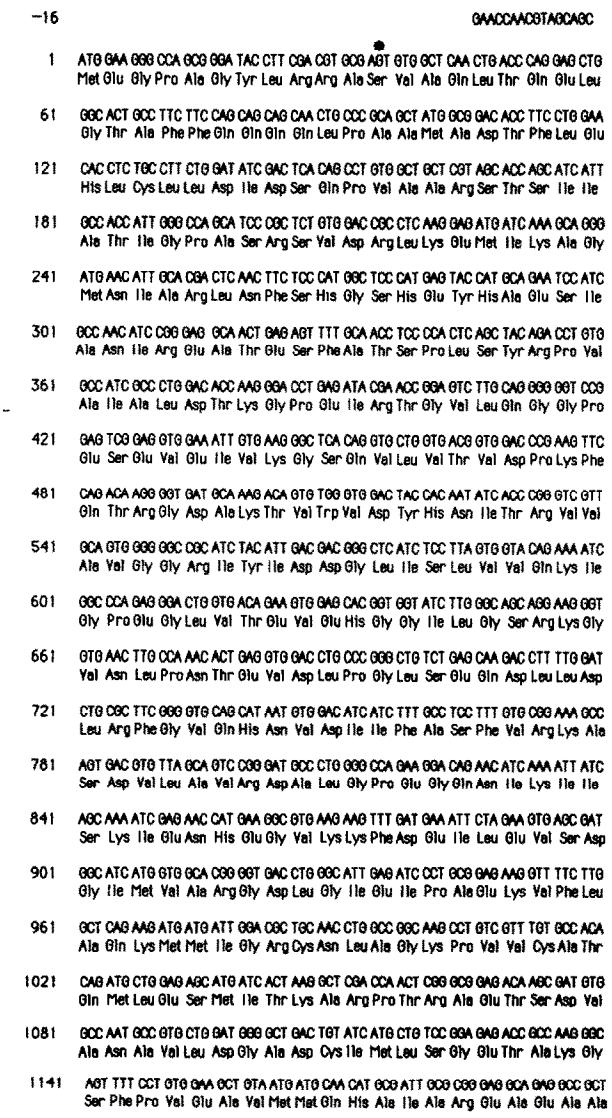
The nucleotide sequence of this insert was determined by the chemical method of Maxam and Gilbert [15] (fig.1). A synthetic 36-mer oligonucleotide complementary to the codons 18–30 (5'-TTGCTGCTGCTGGAAGAAGGCA-GTGCCCAGCTCCTG-3') was synthesized by Dr Igolen, Institut Pasteur de Paris. It was labeled in 5' by polynucleotide kinase [15], annealed to poly(A)-containing mRNA purified from a carbohydrate-fed rat liver, then extended by reverse transcriptase [16]. A 106 base major elongation product was purified by denaturing polyacrylamide gel electrophoresis and sequenced according to Maxam and Gilbert [15].

3. RESULTS AND DISCUSSION

The complete nucleotide and deduced amino acid sequences of PK-L are shown in fig.2. The sequence of 16 5'-non-coding bases and of the first 7 codons was obtained from the primer extension experiment. The 5'-untranslated region seems to be rather short, 19 bases as judged from the length of the primer extension product (106 bases composed of the 36 bases of the primer, 51 first coding bases and 19 5'-non-coding bases). The 1629 coding nucleotides are followed by 1281 3'-untranslated bases and by a poly(A) tail. The canonical AATAAA polyadenylation signal [17] is located 25 bases before the poly(A) tail.

As shown by Saheki et al. [5] the 3'-end of the enzyme is 'Val-Ser', followed by a TGA stop codon. The 3'-untranslated extension contains between positions 2151 and 2225, a sequence complementary to the identifier sequence described by Sutcliffe et al. [1]. The significance of this finding is discussed elsewhere [18]. The phosphorylatable site (Leu-Arg-Arg-Ala-Ser.P.-Val-Ala-Gln) is identical to that reported by Humble [6] and is located close to the 5'-end of the messenger, that is to say close to the N-terminal end of the protein. Since the L and L' subunits were shown to differ by the extremity containing the phosphorylatable site [11], we can assume that L and L' PK subunits have different N-terminal ends. The deduced amino acid sequence reported here fits perfectly with the partial sequence analysis of Hoar et al. [7]. So far the only PKs whose complete amino acid sequence has been determined are chicken muscle [19] and yeast [20] enzymes. Sequence homology between these enzymes and PK-L was 68 and 48%, respectively.

The total length of the PK mRNA whose sequence is reported here is 2930 bases without the poly(A) tail, which fits the 3.2 kb length previously reported for the heavy PK mRNA species. Two shorter, 2 and 2.2 kb long, mRNA species exist; both are polyadenylated and translatable into the same PK-L subunits. Marie et al. (submitted) have



recently demonstrated that these mRNA species differed by their 3′-untranslated extension, probably through a mechanism of alternative splicing.

In conclusion we report the total nucleotide sequence and deduced amino acid sequence of PK-L. This allowed us to locate unambiguously the phosphorylatable site close to the N-terminal end of the protein. In addition PK mRNA exhibits some unusual characteristics, e.g. a very long 3′-untranslated extension and the presence in it of a repetitive sequence complementary to the brain-specific identifier sequence.

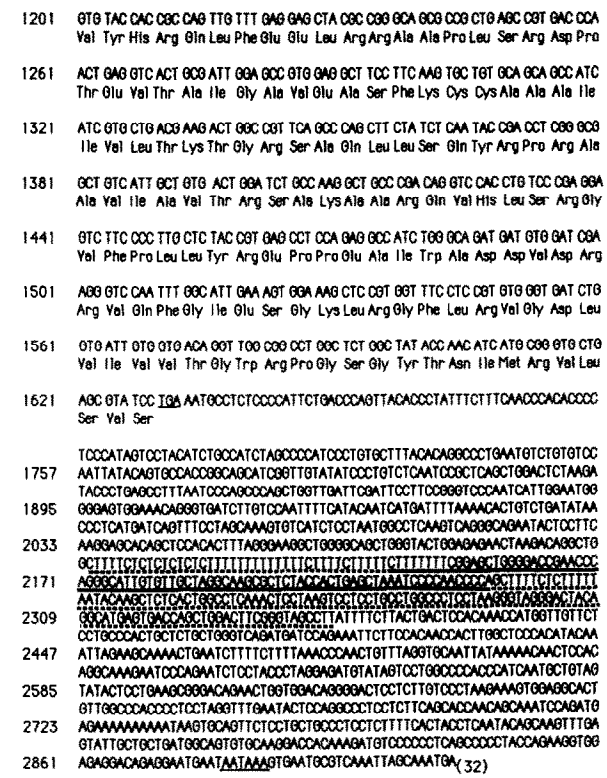


Fig.2. Nucleotide and deduced amino acid sequences of the PK-L mRNA. The canonical AATAAA polyadenylation signal and the TGA stop codon are underlined by a thin line. The cID sequence complementary to the identifier sequence is underlined by a thick line; it is surrounded by 2 Alu-like sequences underlined by a stippled line. Sequence from position −17 to +18 was determined from the primer extension experiment. The phosphorylatable site is indicated by an asterisk.

REFERENCES

[1] Sutcliffe, J.G., Milner, R.J., Bloom, F.E. and Lerner, R.A. (1982) Proc. Natl. Acad. Sci. USA 79, 4942–4946.

[2] Munnich, A., Marie, J., Reach, G., Vaulont, S., Simon, M.P. and Kahn, A. (1984) J. Biol. Chem. 259, 10228–10231.

[3] Ljungström, O., Hjelmquist, G. and Engström, L. (1974) Biochim. Biophys. Acta 358, 289–298.

[4] Riou, J.P., Claus, T.H. and Pilkis, S.J. (1978) J. Biol. Chem. 253, 656–659.

[5] Saheki, S., Saheki, K. and Tanaka, T. (1982) Biochim. Biophys. Acta 704, 484–493.
[6] Humble, E. (1980) Biochim. Biophys. Acta 626, 179–187.
[7] Hoar, C.G., Nicoll, G.W., Schiltz, E., Schmitt, W., Bloxham, D.P., Byford, M.F., Dunbar, B. and Fothergill, L.A. (1984) FEBS Lett. 171, 293–296.
[8] Kahn, A., Marie, J., Garreau, H. and Sprengers, E.D. (1978) Biochim. Biophys. Acta 523, 59–74.
[9] Marie, J., Simon, M.P., Dreyfus, J.C. and Kahn, A. (1981) Nature 292, 70–72.
[10] Saheki, S., Saheki, K. and Tanaka, T. (1978) FEBS Lett. 93, 25–28.
[11] Simon, M.P., Marie, J., Bertrand, O. and Kahn, A. (1982) Biochim. Biophys. Acta 709, 1–8.
[12] Simon, M.P., Besmond, C., Cottreau, D., Chaumet-Riffaud, P., Weber, A., Dreyfus, J.C., Sala-Trepat, J., Marie, J. and Kahn, A. (1983) J. Biol. Chem. 258, 14576–14584.
[13] Noguchi, T., Inove, H., Chen, H.L., Matsubara, K. and Tanaka, T. (1983) J. Biol. Chem. 258, 15220–15223.
[14] Sanger, F., Nicklen, S. and Coulsen, A.R. (1977) Proc. Natl. Acad. Sci. USA 78, 2420–2464.
[15] Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560–564.
[16] Bensi, G., Raugei, G., Klefenz, H. and Cortese, R. (1985) EMBO J. 4, 119–126.
[17] Proudfoot, N.J. and Brownlee, G.G. (1976) Nature 263, 211–214.
[18] Lone, Y.C., Simon, M.P., Kahn, A. and Marie, J. (1985) J. Biol. Chem., in press.
[19] Lonberg, N. and Gilbert, W. (1983) Proc. Natl. Acad. Sci. USA 80, 3661–3665.
[20] Burke, R.L., Tekamp, Olson, P. and Najarian, R. (1982) J. Biol. Chem. 258, 2193–2201.