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Isolation and sequence of a cDNA encoding human platelet phosphofructokinase[1,2]

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A cDNA encoding human platelet 6-phosphofructokinase (PFK; EC 2.7.1.11) has been isolated from a human lymphocyte Raji cell line cDNA library using a cDNA for human muscle PFK as a probe. The platelet cDNA contains 900bp of carboxy terminal coding sequence and 238bp of downstream untranslated region. The deduced amino acid sequence shows 71% identity to the amino acid sequence for the human muscle isoenzyme and 63% identity to the human liver isoenzyme. Almost all of the amino acid residues contributing to catalytic and effector sites in the three isoenzymes are conserved. The platelet gene has been assigned to chromosome 10p15.2-p15.3 by using the cDNA clone as a biotinylated probe against human chromosome spreads (Morrison *et al.* 1991, submitted to Human Genetics).

Mammalian 6-phosphofructokinase (PFK) (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) is a key regulatory enzyme in glycolysis, (Bloxham & Lardy, 1973; Uyeda, 1979). It is a tetrameric enzyme of Mr ~ 340,000 (Dunaway, 1983) which catalyses the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate. There are three human isoenzymes of PFK encoded by the muscle, liver and platelet genes (Vora & Francke, 1981; Vora, 1982). The gene for the muscle enzyme has been sequenced (Nakajima *et al.*, 1987; Sharma *et al.*, 1989) and has been assigned to chromosome 1p32-q32 (Vora, 1982) The gene encoding the liver enzyme has also been sequenced (Levanon *et al.*, 1989; Elson *et al.*, 1990) and has been assigned to chromosome 21q22.3 (van Keuren *et al.*, 1986). The platelet gene has been assigned to chromosome 10p using immunological techniques (Vora *et al.*, 1983) Another putative isoenzyme designated PFKX has been described and localised to chromosome 12 (Ashley *et al.*, 1987). The muscle, liver and platelet isoenzymes are differentially expressed during development and display distinct tissue specificities (Vora, 1982).

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[2] The nucleotide sequence presented here has been submitted to the EMBL/GENBANK database under the accession number M64784.

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Materials and Methods

The cDNA clones for muscle and platelet PFK were isolated from a human lymphocyte cDNA library constructed from a Raji cell line. This library was a kind gift from Dr. Nigel Spurr (ICRF, Clare Hall, London). The cDNA for the muscle isoenzyme was isolated using a genomic fragment encoding human muscle PFK isolated in this laboratory. The cDNA for the muscle isoenzyme was then used as a probe to isolate the cDNA encoding platelet PFK. Approximately $5x10^5$ plaques from the library were screened by plaque hybridisation as described in Sambrook *et al.* (1989). Probe DNA was labelled by random priming (Feinberg & Vogelstein, 1984). The cDNA representing platelet PFK was subcloned into the plasmid pK19 and designated pCS11. Sau3AI restriction fragments derived from the insert in pCS11 were subcloned into the BamHI site of M13mp18 and M13mp19 for DNA sequencing. The insert was also digested with SmaI to give two fragments which were subcloned into the SmaI site of pK19 and Bluescript KS+ (Stratagene™). The various inserts were sequenced by the dideoxy chain termination technique (Sanger *et al.*, 1977). Single stranded M13 phage DNA was isolated by standard procedures (Sambrook *et al.*, 1989), while plasmid DNA for double stranded DNA sequencing was isolated by the alkaline lysis method and purified by CsCl equilibrium density centrifugation (Sambrook *et al.*, 1989). The chromosomal localisation of pCS11 is described elsewhere (Morrison *et al.*, 1991).

The sequencing strategy for the platelet clone is shown in Figure 1.The first sixty bases of the clone were sequenced on one strand only. The remaining bases of coding region were sequenced on both strands.

Results and Discussion

The DNA sequence and inferred amino acid sequence of the platelet PFK clone is shown in Figure 2. The identification of this DNA as that encoding platelet PFK has been inferred from sequence similarities to other PFK sequences, and from its chromosomal location. An alignment of the three human sequences is shown in Figure 3. Eukaryotic PFK is proposed to have arisen as a result of gene duplication and fusion (Poorman *et al.*, 1984) and shows similarities between the amino and carboxyl halves. The amino half is thought to retain catalytic and effector sites similar to the prokaryotic enzymes, while the carboxyl half has evolved to form novel effector sites not found in the smaller prokaryotic enzymes.

Residues important for ligand binding in *Bacillus stearothermophilus* PFK and by implication in the human isoenzymes are shown in Table 1.These residues

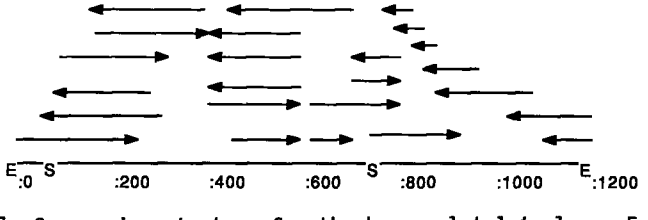

Figure 1. Sequencing strategy for the human platelet clone: E=EcoRI, S=SmaI. The coding region extends from 1 to 900bp. The first 60bp were sequenced on one strand only.

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                                                                    60
ATTCCGAAGTACTTGGAAGAGATCGCCACACAGATGCGCACCAGCATCAACGCGCTGCTG
 I  P  K  Y  L  E  E  I  A  T  Q  M  R  T  S  I  N  A  L  L
                                                                   120
ATCATCGGTGGATTCGAGGCCTACCTGGGACTCCTGGAGCTGTCAGCCGCCCGGGAGAAG
 I  I  G  G  F  E  A  Y  L  G  L  L  E  L  S  A  A  R  E  K
                                                                   180
CACGAGGAGTTCTGTGTCCCCATGGTCATGGTTCCCGCTACTGTGTCCAACAATGTGCCG
 H  E  E  F  C  V  P  M  V  M  V  P  A  T  V  S  N  N  V  P
                                                                   240
GGTTCCGATTTCAGCATCGGGGCAGACACCGCCCTGAACACTATCACCGACACCTGCGAC
 G  S  D  F  S  I  G  A  D  T  A  L  N  T  I  T  D  T  C  D
                                                                   300
CGCATCAAGCAGTCCGCCAGCGGAACCAAGCGGCGCGTGTTCATCATCGAGACCATGGGC
 R  I  K  Q  S  A  S  G  T  K  R  R  V  F  I  I  E  T  M  G
                                                                   360
GGCTACTGTGGCTACCTGGCCAACATGGGGGGGCTCGCGGCCGGAGCTGATGCCGCATAC
 G  Y  C  G  Y  L  A  N  M  G  G  L  A  A  G  A  D  A  A  Y
                                                                   420
ATTTTCGAAGAGCCCTTCGACATCAGGGATCTGCAGTCCAACGTGGAGCACCTGACGGAG
 I  F  E  E  P  F  D  I  R  D  L  Q  S  N  V  E  H  L  T  E
                                                                   480
AAAATGAAGACCACCATCCAGAGAGGCCTTGTGCTCAGAAATGAGAGCTGCAGTGAAAAC
 K  M  K  T  T  I  Q  R  G  L  V  L  R  N  E  S  C  S  E  N
                                                                   540
TACACCACCGACTTCATTTACCAGCTGTATTCAGAAGAGGGCAAAGGCGTGTTTGACTGC
 Y  T  T  D  F  I  Y  Q  L  Y  S  E  E  G  K  G  V  F  D  C
                                                                   600
AGGAAGAACGTGCTGGGTCACATGCAGCAGGGTGGGGCACCCTCTCCATTTGATAGAAAC
 R  K  N  V  L  G  H  M  Q  Q  G  G  A  P  S  P  F  D  R  N
                                                                   660
TTTGGAACCAAAATCTCTGCCAGAGCTATGGAGTGGATCACTGAAAAACTCAAGGAGGCC
 F  G  T  K  I  S  A  R  A  M  E  W  I  T  E  K  L  K  E  A
                                                                   720
CGGGGCAGAGGAAAAAAATTTACCACCGATGATTCCATTTGTGTGCTGGGAATAAGCAAA
 R  G  R  G  K  K  F  T  T  D  D  S  I  C  V  L  G  I  S  K
                                                                   780
AGAAACGTTATTTTTCAACCTGTGGCAGAGCTGAAGAAGCAAACGGATTTTGAGCACAGG
 R  N  V  I  F  Q  P  V  A  E  L  K  K  Q  T  D  F  E  H  R
                                                                   840
ATTCCCAAAGAACAGTGGTGGCTCAAGCTACGGCCCCTCATGAAAATCCTGGCCAAGTAC
 I  P  K  E  Q  W  W  L  K  L  R  P  L  M  K  I  L  A  K  Y
                                                                   900
AAGGCCAGCTATGACGTGTCGGACTCAGGCCAGCTGGAACATGTGCAGCCCTGGAGTGTC
 K  A  S  Y  D  V  S  D  S  G  Q  L  E  H  V  Q  P  W  S  V
                                                                   960
tgacccagtcccgcctgcatgtgcctgcagccaccgtggactgtgtctgtttgtaacact
taagttattttatcagcactttatgcacgtattattgacattgaatacctaatcggcgag
tgcccatctgccccaccagctcccagtgcgtgctgtctgtggagtgtgtctcatgctttc
agatgtgatatgagcagaattaattaaacatttgcctatgAn
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Figure 2. Partial DNA sequenced and inferred amino acid sequence of human platelet phosphofructokinase. The first residue of the sequence corresponds to residue 74 of Bacillus stearothermophilus PFK.

have been identified from crystal structure data for PFK from *E. coli* and *B. stearothermophilus* (Shirakihara & Evans, 1988; Rypniewski & Evans, 1989; Schirmer & Evans, 1990).

The platelet and muscle isoenzymes are more closely related to each other than to the liver isoenzyme, and can be considered to be equally distant from the liver form. The platelet amino acid residues retain 71% and 63% identity to the muscle and liver isoenzymes respectively, while the muscle and liver isoenzymes retain 64% identity.

The platelet clone has been localised to human chromosome 10p15.2-p15.3 in work presented elsewhere (Morrison *et al.*, 1991). Human platelet PFK has previously been assigned to 10pter-p11.1 (Vora *et al.*, 1983). The availability of this sequence should allow the isolation of a full length cDNA clone for the platelet isoenzyme and permit the detailed comparison of all three human isoenzymes.

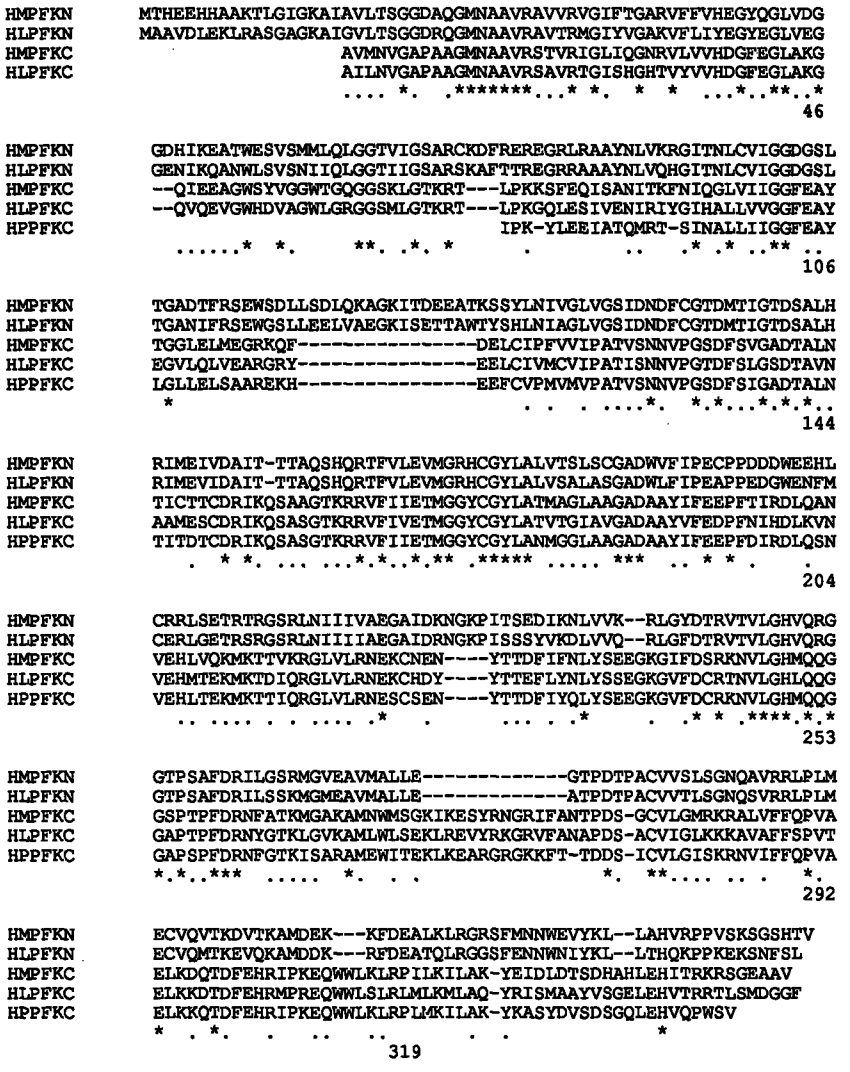

Figure 3. Alignment of the human phosphofructokinases.
*** = identity**
. = similarity
N = AMINO HALF
C = CARBOXYL HALF
HMPFK = HUMAN MUSCLE PFK
HLPFK = HUMAN LIVER PFK
HPPFK = HUMAN PLATELET PFK
Numbering is according to B.stearothermophilus PFK.

Variable expression of the liver, muscle and platelet genes leads to the formation of homo-and heterotetrameric forms of the enzyme with different catalytic and regulatory properties. The least variation in subunit composition occurs in postnatal skeletal muscle which only contains muscle-type subunits. Placenta has predominantly liver-type subunits whereas platelets, brain and fibroblasts have a higher proportion of platelet type subunits (Dunaway *et al.*, 1988; Dunaway & Kasten, 1989).

Table 1. Ligand binding residues from *Bacillus* PFK (Schirmer & Evans, 1990) compared to equivalent residues from human PFK

Residue number	BS	HMN	HLN	HMC	HLC	HPC
A. catalytic site binding F6P and ATP (probably not in carboxyl half)						
11	Gly	Gly	Gly	Gly	Gly	
72	Arg	Arg	Arg	Arg	Arg	
73	Cys	Cys	Ser	Thr	Thr	
103	Asp	Asp	Asp	Phe	Phe	Phe
104	Gly	Gly	Gly	Glu	Glu	Glu
105	Ser	Ser	Ser	Ala	Ala	Ala
125	Thr	Ser	Ser	Thr	Thr	Thr
127	Asp	Asp	Asp	Ser	Ser	Ser
162	Arg	Arg	Arg	Arg	Arg	Arg
243	Arg	Arg	Arg	Arg	Arg	Arg
252	Arg	Arg	Arg	Gln	Gln	Gln
ADP effector site (probably ATP inhibition site in carboxyl half of mammalian PFK)						
21	Arg	Arg	Arg	Arg	Arg	Arg
25	Arg	Arg	Arg	Arg	Arg	Arg
57	Val	Val	Val	Val	Val	Val
154	Arg	Thr	Thr	Lys	Lys	Lys
187	Glu	Asp	Asp	Asp	Asp	Asp
211	Arg	Thr	Thr	Lys	Lys	Lys
213	Lys	Lys	Lys	Lys	Lys	Lys
214	Lys	Lys	Lys	Lys	Lys	Lys
215	His	His	His	His	His	His

Key.N = amino half. C = carboxyl half.
BS-*Bacillus stearothermophilus* PFK.
HM = human muscle PFK
HL = human liver PFK
HP = human platelet PFK

The muscle homotetramer has a higher affinity for fructose 6-phosphate than liver rich isoenzymes from the placenta, kidneys and liver. The presence of high levels of platelet-type subunits in fibroblasts promotes even lower affinities for fructose 6-phosphate. Similarly, muscle homotetramers are less susceptible to inhibition by ATP than isoenzymes rich in liver-type subunits and ATP inhibition becomes more pronounced as the contribution of platelet-type subunit increases in an isoenzyme pool (Dunaway *et al.*, 1988). It has been suggested that the muscle-type subunit will predominate in tissues such as skeletal muscle, heart or brain with

a high rate of glycolysis, whereas the liver-type will predominate in tissues such as liver and kidney where gluconeogenesis is active (Vora, 1982). Dunaway *et al.* (1988) suggest that the platelet-type isoenzyme has an intrinsic enhanced sensitivity to inhibition by ATP. Coupled with a decreased affinity for fructose 6-phosphate, the presence of this isoenzyme could exert a dampening influence on glycolysis and so protect a tissue from excessive lactate accumulation or allow other tissues which are more immediately important for survival to compete more effectively for available glucose.

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