Structure, Distribution, and Functional Expression of the Phosphofructokinase C Isozyme*

Nicholas Gekakis*, Richard C. Johnson*, Ann Jerkins*, Richard E. Main* and Hei Sook Sul†

From the *Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115 and the †Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

To elucidate the structure, tissue-specific expression, and allosteric properties of phosphofructokinase-C (PFK-C), we cloned the cDNA for PFK-C from a rat hypothalamic cDNA library. The cDNA is 2843 base pairs long and encodes a protein of 765 amino acids. The deduced amino acid sequence is homologous to PFK-M (muscle) and PFK-L (liver), 69 and 65% amino acid identity, respectively, especially at substrate binding and catalytic sites, while the allosteric binding sites are less conserved. Tissue-specific expression of PFK-C was investigated by Northern blot analysis. PFK-C mRNA was detected in several brain regions and the anterior pituitary but not in liver, skeletal muscle, or several other tissues. In situ hybridization showed that PFK-C is expressed at a higher level in higher brain regions such as the cortex, compared with the midbrain and basal ganglia, while PFK-L is expressed at approximately equal levels throughout the brain. Expression plasmids containing PFK-C and PFK-L coding sequences were constructed and transfected by transient transfection into CMT cells. Expression of transfected PFKs was demonstrated by PFK enzymatic activity and by Western blotting with anti-rat brain and liver PFK antisera. Allosteric regulatory properties of PFK-C and PFK-L expressed in CMT cells were compared. Fructose 2,6-bisphosphate, a potent activator of PFK, decreased the $K_m$ of PFK-C for fructose 6-phosphate from 200 to 60 μM while decreasing that of PFK-L from 500 to 55 μM. The properties of PFK-C and PFK-L, expressed in CMT cells clearly demonstrate the allosteric differences between the different PFK isozymes.

Phosphofructokinase (PFK) catalyzes the rate-limiting step by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

* This work was supported by National Institutes of Health Grants DK-36264, DK-40518 (to H. S.), DA-00097, and DA-00266 (to R. E. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: PFK, phosphofructokinase; Fru 6-P, fructose 6-phosphate; Fru 2,6-P₂, fructose 2,6-bisphosphate; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; TES, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; RT-PCR, reverse transcription-polymerase chain reaction; PFK-C, phosphofructokinase-C; bp, base pair(s); PFK-M, muscle; PFK-L, liver; rPFK-C, rat PFK-C; mPFK-L, mouse PFK-L; mPFK-M, mouse PFK-M; TES, 1,4-piperazinediethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis.

and committed step in glycolysis, the conversion of fructose 6-phosphate (Fru 6-P) to fructose 1,6-bisphosphate. The enzyme is subject to allosteric regulation by numerous effectors, which serve to integrate glycolysis with other cellular activities (1). Enzymatically active PFK is a tetramer or higher order oligomer with a subunit molecular mass of 85 kDa (2). Three subunit isoforms of PFK have been identified, which form homo- and heterotetramers with differing catalytic and allosteric properties (3). PFK-M is specific for cardiac and skeletal muscle. PFK-L is expressed in many tissues but is most abundant in the liver. PFK-C is expressed in several tissues including brain; however, it is not known if PFK-C is specific to any particular cell types within the brain.

PFK has been extensively studied for many years. However, most of our understanding of mammalian PFK structure and function comes from the PFK isoforms expressed in liver and muscle, PFK-L and PFK-M, respectively. Relatively little is known about the third PFK isozyme, expressed in brain, PFK-C. The cDNAs for mouse PFK-L (4) and mouse, rabbit, and human PFK-M (5-7) have previously been cloned and characterized. The different isoforms of PFK respond differently to allosteric effectors, so the tissue-specific pattern of PFK isozyme expression determines how PFK activity, and therefore glycolysis, is regulated in a particular tissue. Muscle PFK is composed almost entirely of the M isozyme (PFK-M), and its allosteric properties have been extensively studied (1). The properties of PFK-L are well understood as liver PFK is composed of the tetramers PFK-L,M and PFK-L,L (3). However, PFK-C is not exclusively expressed in any tissue and its allosteric properties are not well known.

In what tissues is PFK-C expressed and how does its expression affect the regulation of glycolysis in those tissues? Unlike PFK-M and PFK-L, the cDNA for PFK-C has never been cloned from any organism, and there is little data on its primary structure. There are several reports on the regulation of brain PFK in mammals (8-10), but in only one case has pure PFK-C been isolated and studied (11). We therefore cloned PFK-C from a rat hypothalamic cDNA library in an effort to answer these questions. The entire coding sequence as well as 3'- and 5'-untranslated regions were sequenced. By comparing the deduced amino acid sequence of rat PFK-C (rPFK-C) with those of mouse PFK-L (mPFK-L) and mouse PFK-M (mPFK-M), which we have previously cloned (4, 5), we show that the substrate binding and catalytic sites are highly conserved, while the allosteric binding sites are less conserved. We next examined the tissue distribution of PFK-C by Northern blot analysis. PFK-C is present in several brain regions and the anterior pituitary but not in liver or several other tissues. To study the allosteric regulatory properties of PFK-C, we have expressed PFK-C coding sequences in CMT cells. The presence of PFK-C in transfected cells was demonstrated by immunoreactivity with PFK-C-specific an-
tibody in a Western blot. Cells transfected with PFK-C expression plasmid had a 4-fold higher PFK activity than control, untransfected cells. Fructose 2,6-bisphosphate (Fru 2,6-P₂) was not significantly altered in PFK-L-expressing cells, an important allosteric effector of PFK, activated PFK-C expressed in CMT cells by lowering its Kₘ for Fru 2,6-P₂. However, Fru 2,6-P₂ lowered the Kₘ of PFK-C expressed in CMT cells for Fru 6-P to a lesser extent than it lowered that of PFK-L for substrate.

**Experimental Procedures**

**cDNA Cloning**—We initially cloned rPFK-C cDNA sequence by oligo(dT) primed RT-PCR using hippocampal RNA during an attempt to clone prohormone convertase cDNA sequences. The primers were synthesized to be degenerate sequences corresponding to the highly conserved regions in rat prohormone convertase 1 (nucleotides 1007-1451), prohormone convertase 2 (nucleotides 1109-1556), and furin (nucleotides 1187-1631) (12). The sense primer was 5'-TACA-GYGGMACGTCGGGCC-C3' and the antisense primer was 5'-CAGRTGTYTCATRPTYCCKCA-3'. The 365-bp RT-PCR product was gel-purified, reamplified, subcloned into pcDNA3 (Strategene, LaJolla, CA), and used to screen 2 × 10⁸ plaque-forming units from a rat hypothalamic cDNA library. Four positive clones were isolated and further plaque-purified (12, 13). None of the original four clones represented the full-length PFK-C cDNA sequence, so the library was rescreened with a 317-bp restriction fragment representing the 5' untranslated region and with a 23-base synthetic oligonucleotide, which was specific for PFK-C cDNA (Fig. 1, bp 459-481). pcDNA3 containing the full-length cDNA clone was in vitro excised from the Lambda ZapII (Strategene) clone according to the manufacturer's protocol. The resulting pcDNA3 II SK+ plasmid was sequenced by the dideoxy chain termination method (14) using universal and specific synthetic oligonucleotide primers.

**Northern Blot Analysis**—Total RNA was prepared from various rat tissues by homogenization in guanidinium isothiocyanate followed by CsCl centrifugation (15). 10 μg of total RNA was fractionated by electrophoresis through a 0.7% agarose, 2.2 M formaldehyde gel, and autoradiographed with an intensifying screen. Equal loading of RNA was verified by ethidium bromide staining of 18 S ribosomal band.

**In Situ Hybridization**—The in situ analyses were performed as described (16). Riboprobes corresponding to the 5' regions (191-248 nucleotides) of PFK-L, PFK-M, and rat PFK-C were generated using [³²P]UTP in an in vitro transcription reaction to create the antisense probes; as a control, the same region of PFK-C was also prepared as a sense probe. Because the mouse and rat PFK-L sequences are >95% identical at the nucleotide level in most of this region, it was expected that the mouse probes would work well on rat tissue. The 5' regions were used because there is no homology among the sequences of the three different isoforms. Sagittal and coronal sections were hybridized to the riboprobes and exposed to x-ray film for 4 days.

**Expression of PFK Isozymes in CMT Cells**—All restriction enzymes were provided by New England Biolabs (Beverly, MA). The mammalian expression vector, pcDNAI (Invitrogen), was used to express PFK cDNAs in CMT cells in culture. To clone rPFK-C cDNA into pcDNAI, pcDNA3 II SK+ containing the full-length rPFK-C cDNA was cut with HindIII, in the multiple-cloning site upstream of the insert and with AvaII at base 2400 in the 5'-untranslated region. The 2.4-kb HindIII/AvaII fragment was inserted into HindIII/Xbal-cut pcDNAI downstream of the cytomegavirus promoter. To clone mPFK-L cDNA into pcDNAI, pGEM-4Z containing the full-length mPFK-L cDNA (4) was cut with EcoRI in the multiple-cloning site upstream of the insert and with Nhel at base 2439 in the 3'-untranslated region. The 2.4-kb EcoRI/Nhel fragment was inserted into EcoRI/Xbal-cut pcDNAI.

The pcDNAI constructions, which contain the SV40 origin of replication, were transfected into CMT cells (17), which are COS cells modified with SV40 large T antigen under the control of the mouse metallothionin promoter. We chose the CMT line for these studies because we found substantial signals in the hypothalamus, hippocampus, cortex, and cerebellum; very weak signals were found in submaxillary gland, liver, and kidney (data not shown). A GenBank search showed high homology of the 3'-120 bp sequence of this clone to PFK-L (4) and PFK-M (6). Screening a rat hypothalamic cDNA library with the RT-PCR sequence yielded several additional PFK-C cDNA clones. The 5' 240 nucleotides of the RT-PCR clone were amplified in any other brain PFK cDNA clones and showed no significant homologies to known sequences in GenBank. We therefore conclude that the unrelated 240-nucleotide sequence is an artifact of PCR. The full-length cDNA sequence and deduced amino acid sequence obtained are shown in Fig. 1. The cDNA is 2643 bp, which includes 2298 bp of coding sequence, 63 bp of 5'-untranslated region, and 281 bp of 3'-untranslated region. The encoded protein is 765 amino acids, 15 amino acids fewer than either PFK-L or PFK-M (4, 6) and...
FIG. 1. Sequence of rat PFK-C. The full-length PFK-C cDNA sequence and deduced amino acid sequence is shown. Several overlapping clones of PFK-C cDNA were sequenced in both directions by the dideoxy chain termination method using universal and specific synthetic oligonucleotide primers. The top line is nucleotide sequence and is numbered at the left. The deduced amino acid sequence is shown below in boldface. The translation initiation codon is at 64-66, and a stop codon is at 2359-2361.
Phosphofructokinase C

FIG. 1.—continued
has a calculated molecular mass of 82 kDa. While the coding sequences of mPFK-L, mPFK-M, and rPFK-C cDNAs are highly homologous, there is no similarity among the 5'- and 3'-untranslated regions of these three cDNAs (data not shown). However, there is significant homology between 3'-untranslated region of mouse and human PFK-M. As with the coding sequences, there is greater homology in the 3'-untranslated region between the same isozyme of different species than between different isozymes in the same species. The antisense primer contained a 14/16 nucleotide match with PFK-C cDNA sequence from 1772-1787.

Comparison of the Primary Structure of the Phosphofructokinase Isozymes—The amino acid sequence alignment of rat PFK-C, mouse PFK-M, and mouse PFK-L is shown in Fig. 2. Overall there is high homology among the three enzymes with a 69% amino acid identity between the M and C isozymes, 65% between L and C, and 68% between M and L suggesting a strong evolutionary pressure to conserve structural elements. Poorman et al. (23) have postulated that mammalian PFK arose by gene duplication of an ancestral PFK structurally similar to bacterial PFK. The amino- and carboxy-terminal halves of rabbit PFK-M are homologous to each other and to PFK from Bacillus stearothermophilus. The NH2-terminal half is more evolutionarily conserved than the COOH-terminal half, leading to the hypothesis that the NH2-terminal half of the protein is the catalytic domain, while the COOH-terminal half contains allosteric binding sites. In support of this hypothesis, the three mammalian PFK isozymes are more closely related in the NH2-terminal half (amino acids 5-371, 74% identity between L and C) than in the COOH-terminal half (amino acids 403-745, 64% identity between L and C).

Tissue Distribution of Phosphofructokinase C mRNA—The expression of PFK-C mRNA was examined in a number of rat tissues using a specific cDNA probe from the 3'-untranslated region. Strong signals were found in several brain regions and the anterior pituitary as shown in Fig. 3. PFK-C mRNA was also present in the neurointermediate lobe of the pituitary (data not shown). A very low level of PFK-C mRNA was detected in ventricle and lung. No signal was detected in skeletal muscle, liver, and several other tissues. These results indicate that PFK-C mRNA is expressed at a high level mainly in neuroendocrine tissues.

Comparison of PFK-C, PFK-L, and PFK-M mRNA Expression in Rat Brain—In situ hybridization of rat brain sagittal sections (Fig. 4) show that PFK-L mRNA (L) is present in all areas of the brain, while PFK-M (M) and PFK-C (C) are specific for certain areas of the brain. The brain sense probe (Sense), used as a control, showed no detectable binding to the sections. PFK-C was much less abundant in the midbrain (MB) and the basal ganglia (BG) than was PFK-M, as also seen in the coronal section in the bottom right of Fig. 4. PFK-C was most abundant in the cortex (Cx), hippocampus (H), cerebellum (Cb), and olfactory bulb (OB). These results agree with those of the Northern blot shown in Fig. 3 and with those from the original Northern blot carried out using the sequence obtained from RT-PCR (data not shown). Thus PFK-C mRNA is expressed at relatively higher levels in higher brain regions, while PFK-M mRNA is present in virtually all areas rich in nerve cell bodies.

Expression of PFK-C and PFK-L in CMT Cells—Our goal was to understand how the presence of PFK-C affects the regulation of glycolysis in the tissues in which it is expressed. To study the function of PFK-C, we expressed PFK-C in CMT cells in culture. The coding regions of PFK-C and PFK-L were cloned into a mammalian expression vector and transiently transfected into CMT cells. After harvesting and lysing the cells, cell extracts were assayed for PFK activity. Table I shows the PFK activity in cell extract of CMT cells transfected with various plasmids. Extracts of CMT cells transfected with PFK-C expression plasmid have a PFK activity 4-fold higher than the control extracts from cells transfected with vector alone. Extracts from mock-transfected CMT cells had a PFK activity approximately equal to that from cells transfected with nonrecombinant vector. Extracts from cells transfected with PFK-L had an activity 5-fold higher than control.

The expression of PFK isoforms by transfection was also analyzed by Western blot. Assignment of the PFK-L and PFK-C bands was made by first probing the blots with isoform-specific antisera (data not shown) and by the relative mass of the different PFK isoforms. The Western blot in Fig. 5 was probed with antiserum raised against PFK purified from rat brain, which reacts with all three isoforms. This antiserum also cross-reacts with several cellular proteins as shown by the bands in lanes 1 and 2, which contain extracts from mock-transfected cells or cells transfected with vector alone, respectively. In addition to these nonspecific bands, lanes 1 and 2 also have a faint band at approximately 82 kDa, which corresponds to PFK-C, indicating that CMT cells express PFK-C. Extracts from cells transfected with PFK-L expression plasmid (lane 3) show a specific band corresponding to PFK-L (arrow) in addition to the nonspecific bands in lanes 1 and 2. Extracts from cells transfected with PFK-C expression plasmid (lane 4) contain a band corresponding to PFK-C (arrow), at much greater intensity than the corresponding band in lanes 1 or 2. Although PFK-C actually has a lower calculated molecular weight than PFK-L, we (5) and others (9) have shown that PFK-C runs with a higher apparent molecular weight than PFK-L on SDS-PAGE.

Allosteric Regulation of PFK-C and PFK-L—CMT cells transfected with PFK-C expression plasmid have a PFK activity 4-fold higher than background and have an immunoreactive level of PFK-C much higher than that in control cells. We conclude that most of the PFK activity assayed in extracts from cells transfected with PFK-C expression plasmid is due to the transiently expressed protein and that the active form of PFK in this extract is mostly PFK-C. Therefore, the regulation of PFK activity in extracts from transfected cells largely reflects the properties of PFK-C.

Fru 2,6-P2 is a potent allosteric activator of PFK. PFK-L has been reported (11) to be more sensitive to activation by Fru 2,6-P2 than PFK-C. We examined the response of PFK-L and PFK-C in CMT cells (Fig. 6) by assaying the effect of Fru 2,6-P2, which is an intermediate substrate in the active form of PFK in this extract is mostly PFK-C. Therefore, the regulation of PFK activity in extracts from transfected cells largely reflects the properties of PFK-C.

Fru 2,6-P2 is a potent allosteric activator of PFK. PFK-L has been reported (11) to be more sensitive to activation by Fru 2,6-P2 than PFK-C. We examined the response of PFK-L and PFK-C in CMT cells (Fig. 6) by assaying the effect of Fru 2,6-P2, which is an intermediate substrate for PFK-C expression plasmid. At the lowest (0.04 mM) and highest (2.0 mM) concentrations of Fru 6-P, PFK-C activity was similar in the absence or presence of 50 μM Fru 2,6-P2. However, at an intermediate substrate concentration (0.1 mM), PFK-C activity was 25% of Vmax in the absence and 63% of Vmax in the presence of activator. Similarly, at 0.04 mM Fru 6-P, PFK-L activity was the same in the absence or presence of activator. However, at higher concentrations of substrate, PFK-L was much more active in the presence of 50 μM Fru 2,6-P2. A Lineweaver-Burk plot of these data showed the Km of PFK-C for Fru 6-P to be 200 μM in the absence of Fru 2,6-P2, and 60 μM in its presence, while Fru 2,6-P2 lowered the Km of PFK-L for Fru 6-P from 300 to 55 μM.
Phosphofructokinase

Fig. 2. Comparison of the deduced amino acid sequences of rat PFK-C and mouse PFK-L and PFK-M. The complete amino acid sequence of rPFK-C is shown. Below are the sequences for mPFK-L and mPFK-M, which are compared with it. Dashes represent a residue identical to that of rPFK-C; nonidentical, aligned amino acids are shown in capital letters; nonaligned amino acids are lower case letters, and gaps are represented as dots. The numbering is with respect to rPFK-C amino acid sequence. The computer-generated alignment was done according to the method of Feng and Doolittle (29).

DISCUSSION

To begin to understand the structure/function relationship of the PFK-C isozyme, we cloned, sequenced, and expressed in vitro PFK-C cDNA from rat brain. In the only other report of PFK-C structure, Valaitis et al. (20) sequenced phosphopeptides resulting from V-8 protease digestion of PFK from rabbit brain following in vitro phosphorylation. Four phosphopeptides 11-13 amino acids long were isolated and sequenced. One was identical to the previously published (6) mPFK-L-deduced amino acid sequence at the 12 carboxyl-terminal residues. Two of the phosphopeptides were identical to mPFK-L-deduced amino acid sequence in 11 of
Phosphofructokinase C

FIG. 3. Northern blot analysis of PFK isozyme distribution. Total RNA (10 μg/lane) from the indicated rat tissues was analyzed on a denaturing gel, transferred to Nytran (Schleicher and Schuell), and probed with a random primed cDNA for nucleotides 2395–2643 of rat PFK-C in SDS-PIPES (12). The blot was exposed to film for 22 h. The abbreviations for the tissues are: HY, hypothalamus; OB, olfactory bulb; Cb, cerebellum; PC, parietal cortex; Hi, hippocampus; Ve, ventricle; SM, skeletal muscle; Lu, lung; Li, liver; K, kidney; Sp, spleen; D, duodenum; Ov, ovary; T, testes; Su, submaxillary gland; AP, anterior pituitary. Similar results were obtained with an independent set of tissue RNAs using a nearly full-length probe, including a positive signal in the neurointermediate lobe of the pituitary.

FIG. 4. Analysis of PFK isozyme mRNA distribution in rat brain by in situ hybridization. Sagittal and coronal sections were hybridized with antisense riboprobes from the 3’ regions of mouse PFK-L (L), mouse PFK-M (M), or rat PFK-C (B) PFK. Control sense (Sense) probe was made to the same region of rat brain PFK. The sections were exposed for 4 days. Four different sets of sections were analyzed with four different sets of probes with similar results. BG, basal ganglia, Cb, cerebellum, Cx, cortex, H, hippocampus, MB, midbrain, OB, olfactory bulb.

13 or 9 of 11 positions. Again, this sequence was at the carboxyl terminus of the deduced amino acid sequence. The fourth phosphopeptide was presumed to contain sequence of rabbit PFK-C and was thought to be at either the amino or carboxyl terminus because protease digestion led to only a slight decrease in M, of PFK-C as demonstrated by SDS-PAGE. However, our deduced amino acid sequence of rat PFK-C shows no similarity to the rabbit PFK-C phosphopep-

TABLE I

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>PFK activity</th>
<th>ΔA280 in/min/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFK-C</td>
<td>5.38</td>
<td></td>
</tr>
<tr>
<td>PFK-L</td>
<td>6.95</td>
<td></td>
</tr>
<tr>
<td>Vector</td>
<td>1.30</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 5. Western blot of extract from CMT cells transfected with PFK expression plasmids. CMT cells were transfected and extract was prepared as described under “Experimental Procedures.” Extract was fractionated on 6% SDS-PAGE and transferred to Immobilon polyvinylidine difluoride, which was probed with antibody to rat brain PFK. Horseradish peroxidase-conjugated goat anti-rabbit IgG was used as a second antibody. Assignment of bands was made based on the results of Western blots done with isozyme-specific antibody. Lane 1, untransfected cell extract; lane 2, extract from cells transfected with vector containing no insert; lane 3, extract from cells transfected with PFK-L expression plasmid; lane 4, extract from cells transfected with PFKC expression plasmid.

A.

B.
Phosphofructokinase C

3355

tide sequence at the carboxyl or amino terminus. There is, however, a 6 of 15 amino acid match of this phosphopeptide at positions 204–216 of rat PFK-C deduced amino acid sequence. The phosphopeptide may represent another region of rabbit PFK-C or may not derive from PFK-C at all.

PFK-C CDNA is similar in size to both PFK-L and PFK-M. However, the deduced amino acid sequence of PFK-C is 15 amino acids shorter than either PFK-L or PFK-M due to a truncation at the amino terminus. We have sequenced four independent PFK-C CDNA clones that show the same nucleotide sequence in this region. Moreover, there is no other upstream ATG present in the 5′-untranslated region. Furthermore, expression of this CDNA sequence in CMT cells generated a protein with the same mobility as the endogenous PFK-C. These results support our assignment of the translation initiation codon. Amino-terminal sequence analysis of PFK-C protein will help to verify our assignment of the translation initiation codon. Significantly, the truncation of PFK-C is in a region where PFK-M and PFK-L sequences diverge considerably. Elsewhere there are evolutionarily conserved regions among the three mammalian PFK isozymes. Evolutionary conservation is especially strong in the substrate binding and catalytic sites, while allosteric sites are more diverged. X-ray crystallography (24, 25) and site-directed mutagenesis (26) have identified Arg-252 of PFK-M in that it has high affinity for its substrate, Fru 6-P, in the absence of any allosteric effector, while PFK-L requires the presence of Fru 2,6-P2 for activity under physiological conditions (22). PFK-L’s dependence on Fru 2,6-P2 for activity allows it to be hormonally regulated; therefore glycolysis in the liver is integrated with the energy status of the whole organism. Brain glycolysis is not known to be hormonally regulated, and brain PFK activity is normally independent of allosteric effectors. While PFK-L is present in brain, its dependence on allosteric activators for activity may be diminished by its association with PFK-M and PFK-C.

In summary, we cloned the cDNA for rat PFK-C mRNA to initiate studies on PFK-C gene regulation and structure/function relationships of the PFK isozymes. Overall, the amino acid sequence of PFK-C, PFK-M, and PFK-L is highly conserved (23); except for the COOH-terminal residue is in the middle of a stretch of 23 amino acids, 22 of which are identical among the M, L, and C isoforms (Fig. 2). Asp-127 of the major Escherichia coli PFK, which corresponds to Asp-154 of PFK-C (23), is an important residue in the phosphoryl transfer reaction (27) and is part of a highly conserved region in which 24 consecutive amino acids are identical among the three isozymes (Fig. 2). Valaitis et al. (28) showed that removal of the 17 COOH-terminal amino acids of PFK from rabbit muscle reduced its sensitivity to ATP inhibition. In this region 7 of the 17 amino acids are conserved between M and L isoforms, while only four of the 17 are conserved between M and C. Therefore, the less stringent evolutionary conservation in this region may reflect the differences of the various isoforms in their sensitivity to inhibition by ATP.

Results of our Northern blots show that PFK-C is most abundant in neuroendocrine tissues. Our previous results (4) showed that PFK-L is expressed in a wide range of tissues, and PFK-M is expressed in muscle and brain. We conclude that PFK-L is widely expressed in mouse tissue and fills the requirements of the various isozymes in their sensitivity to inhibition by ATP. Muscle requires the rapid production of ATP at the onset of rapid contraction and sustained glucose oxidation during aerobic exercise. PFK-M is presumably specialized to meet these requirements due to its lower sensitivity to inhibition by ATP, its greater affinity for Fru 6-P in the absence of any allosteric activator (9), and its higher specific activity (1).

PFK-C is less sensitive than PFK-L to inhibition by Fru 2,6-P2. Decreased activation is due to a higher activity of PFK-C in the absence of activator and a lower activity in the presence of activator. To study the allosteric regulation of PFK-C, Kemp and Foe (11) purified PFK from rat brain, dissociated the heterotetramers, isolated the C subunit, and reconstituted the subunits to form active tetramers. In this way they showed that the concentration of Fru 2,6-P2 required for 50% activation of PFK-L and PFK-C was 50 nM and 4.5 μM, respectively. On the other hand, Ishikawa et al. (21) reported approximately equivalent sensitivity to Fru 2,6-P2 of liver and brain PFK. However, the brain PFK preparation employed in the latter study probably contained hybrids of PFK-M, PFK-L, or PFK-C, not purified isoform (11). PFK-C is like PFK-M in that it has high affinity for its substrate, Fru 6-P, in the absence of any allosteric effector, while PFK-L requires the presence of Fru 2,6-P2 for activity under physiological conditions (22). PFK-L’s dependence on Fru 2,6-P2 for activity allows it to be hormonally regulated; therefore glycolysis in the liver is integrated with the energy status of the whole organism. Brain glycolysis is not known to be hormonally regulated, and brain PFK activity is normally independent of allosteric effectors. While PFK-L is present in brain, its dependence on allosteric activators for activity may be diminished by its association with PFK-M and PFK-C.

Acknowledgments—We thank Dr. Robert G. Kemp for anti-rat brain PFK and anti-PFK-C antiserum, Drs. Mark Ferrella and Cesario Bianchi for help with CMT cell transfection, and Dr. Shaw Pang Yet for help with Western blotting. We also thank Drs. Betty Eipper and Retan Bhat for help with the in situ hybridization analyses and Dr. Brian Bloomquist for advice.

REFERENCES


3505