Vol. 165, No. 3, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 29, 1989 Pages 1345-1351

SEQUENCE OF RAT SKELETAL MUSCLE PHOSPHOGLYCERATE MUTASE cDNA

J. Castellà-Escolà,[1] L. Montoliu,[2] G. Pons,[1]
P. Puigdomènech,[2] M. Cohen-Solal,[3] J. Carreras,[1]
J. Rigau,[2] and F. Climent[1]*

[1]Unitat de Bioquímica, Facultat de Medicina, Universitat de Barcelona, Casanova 143, 08036 Barcelona, Spain

[2]Departament de Genètica Molecular, C.I.D., Consejo Superior de Investigaciones Científicas, Jordi Girona Salgado 18, 08034 Barcelona, Spain

[3]INSERM U-91, Hôpital Henri Mondor, 94010 Créteil, France

Received November 13, 1989

A cDNA clone coding rat skeletal muscle phosphoglycerate mutase was isolated from a rat muscle lambda gt10 cDNA library and its sequence was determined. The deduced protein possesses 252 amino acids and is 94% homologous with respect to human muscle phosphoglycerate mutase. No amino acids changes occur at the active site and structural predictions suggest strong conformational homologies with other enzymes of the mutase family.

Phosphoglycerate mutases are a family of enzymes which catalyze the transfer of phosphoryl groups among carbons of phosphoglycerates (see reference 1 for a recent review). In mammalian tissues these enzymes are multifunctional and possess three different enzymatic activities in different proportions: phosphoglycerate mutase (Gri-3-P + Gri-2,3-P_2 <----> Gri-2-P + Gri-2,3-P_2); 2,3-bisphosphoglycerate synthase (Gri-1,3-P_2 + Gri-3-P <----> Gri-2,3-P_2 + Gri-3-P) and 2,3-bisphosphoglycerate phosphatase (Gri-2,3-P_2 ----> Gri-3P + Pi). The forms which show relatively higher mutase activity are considered the phosphoglycerate mutase (PGAM, E.C. 5.4.2.1) isozymes, and those which show increased

* To whom correspondence should be addressed.

Abbreviations used:
PGAM, phosphoglycerate mutase; PGAM-M, muscle-specific isozyme of PGAM; PGAM-B, brain type isozyme of PGAM; BPGM, bisphosphoglycerate mutase; Gri-2,3-P_2, 2,3-bisphosphoglycerate; Gri-3-P, glycerate-3-phosphate; Gri-2-P, glycerate-2-phosphate; Gri-1,3-P_2, 1,3-bisphosphoglycerate.

0006-291X/89 $1.50

synthase and phosphatase activity correspond to the bisphosphoglycerate synthase/phosphatase or bisphosphoglycerate mutase (BPGM, E.C. 2.7.5.4).

In mammals, PGAM requires Gri-2,3-P_2 as a cofactor and is present in three isozymic forms, resulting from homo- and hetero-dimeric combinations of two different subunits, types M and B (2), whose cDNAs have recently been cloned from human skeletal muscle (M Type) (3) and brain (B Type) (4). Both M and B subunits are 80% homologous in their amino acid sequence, and possess similar immunological properties (5). Both subunits are encoded by two different genes; one, the human M gene, has also been isolated recently (6). BPGM is found mainly in erythrocytes (7, 8) and is also present in a dimeric form resulting from the combination of a type E subunit with, respectively, another type E subunit, or with a type M or type B, with the EE form being the most abundant (9). The E subunit has also been cloned from human (10), rabbit (11) and mouse erythrocytes (12). Their amino acid sequences are 50% homologous with respect to human PGAM subunits. PGAM is not restricted to mammals but is present in all other organisms of which the best known is yeast. This enzyme is a cofactor-dependent PGAM which, in active form, is a homotetramer whose corresponding gene has also been cloned and sequenced (13). In some organisms, such as plants, there exists a non cofactor-dependent enzyme instead of the Gri-3-P dependent PGAM. The cofactor-independent is a monomer which lacks BPGM activity (14) but little structural information on this mutase is available at present.

Despite information on human PGAM, little is known of the enzyme from rat tissue. In our laboratory, differentiation of rat tissues with emphasis on myogenesis is being studied (15). With the aim of performing such experiments, we present here cloning and sequencing of rat skeletal muscle cDNA with the entire coding region of PGAM-M. The great homology in amino acid sequence and tertiary structure in the PGAM family is also reported.

MATERIALS AND METHODS

A rat soleus muscle cDNA library (kindly supplied by Dr. B. Nadal-Ginard, Boston) made in lambda gt10 was screened with a 480 bp fragment, derived from the Nco I cleavage of the human skeletal muscle phosphoglycerate mutase gene, corresponding to the amino terminus of the deduced protein sequence (6). Phage plates from 10^5 recombinants were transferred to nitrocellulose filters and were hybridized in 5xSSC, 5xDenhard's, 50% formamide, 25 mM sodium phosphate, 0.1% SDS, 5 mM EDTA, 250 µg/ml of denatured salmon sperm DNA and the [^{32}P] random primed labelled probe at 42°C for 24 h. Filters were washed 4 times in SSC buffer containing 0.1% SDS with the stringency being increased up to 0.2xSSC at 65°C. Seventeen positive clones were rescreened until all plaques gave a positive signal with the probe. After two sets of rescreening, 8 positive clones were finally confirmed. DNA from these clones was amplified and purified by the method described by Davis et al. (16) and the inserts were isolated by digestion with Eco RI. One of the inserts, 820 bp long, which corresponds to the full-length PGAM-M cDNA was subcloned into pUC 19. This insert was subcloned into M13 mp18, cleaved with Bgl II and the resulting fragments were subcloned again in M13 mp18. The fragments were

sequenced in duplicate in both orientations by the dideoxy method of Sanger (17). Sequence analysis and further studies were performed using the Microgenie programme (Beckman Corp.).

RESULTS AND DISCUSSION

The probe used in this work was derived from the 5' translated region of the human gene (6). In order to ascertain the suitability of the probe for our cloning purposes, Southern blots of rat genomic DNA were hybridized with the human probe. Under high stringency conditions, good hybridization was observed (data not shown). By using either the same probe or oligonucleotide probes synthesized from human PGAM-M sequence, a mRNA size of approximately 800 bp was determined for rat muscle M subunit (Figure 1). Figure 2 shows the sequencing strategy for rat PGAM-M cDNA and its restriction map. The nucleotide sequence of the coding region and the deduced amino acid sequence are shown in figure 3, and in figure 4 the aminoacid alignment with other related enzymes is presented.

Rat skeletal muscle PGAM-M cDNA is 87% homologous to that of human muscle, a value that is the same as that of human, rabbit and mouse BPGM, a related enzyme. Many base changes take place in the third position of codons and in many cases imply no changes in the aminoacid sequence. Thus, amino acid homology is greater (94%) than the

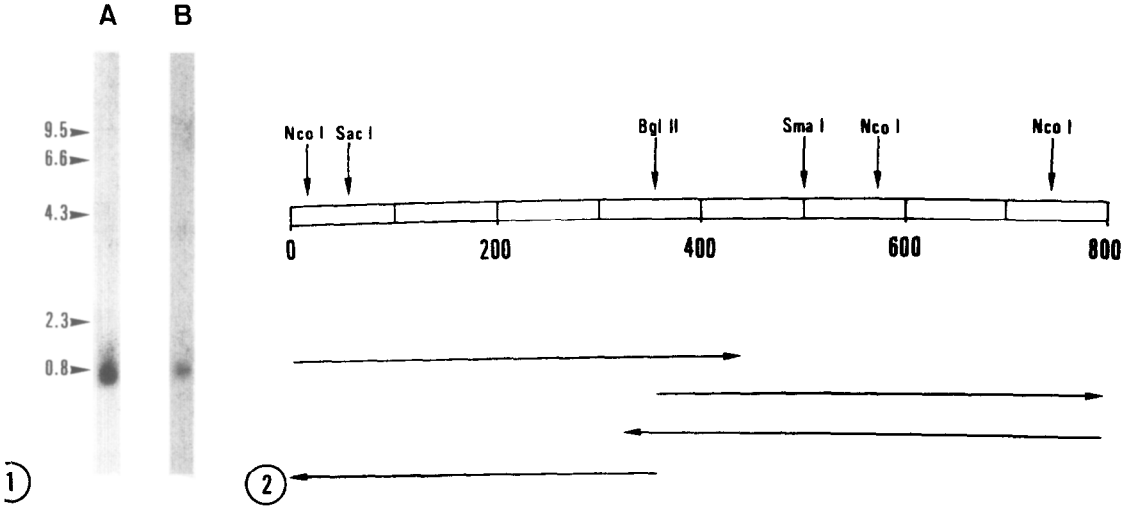

Figure 1
Northern blot analysis of rat PGAM-M mRNA hybridized with (A) human probe and (B) oligonucleotide probe (see Results). Arrows indicate the size of the standards (kb).

Figure 2
Strategy for the sequencing and restriction map of rat PGAM-M cDNA. Arrows indicate the direction and the extend of each sequence obtained after sub-cloning into M13.

```
                                                                    62
   cgcgtcccctgcccacc ATG GCC ACC CAC CGC CTA GTA ATG GTC CGC CAC GGT GAG AGC TCA
                      M   A   T   H   R   L   V   M   V   R   H   G   E   S   S

                                                                   122
TGG AAC CAA GAG AAC CGT TTC TGT GGC TGG TTT GAT GCA GAA CTG AGT GAG AAG GGG GCA
 W   N   Q   E   N   R   F   C   G   W   F   D   A   E   L   S   E   K   G   A

                                                                   182
GAG GAG GCC AAG CGG GGG GCC ACT GCC ATC AAA GAT GCC AAG ATA GAG TTT GAC ATC TGC

                                                                   242
TAC ACG TCG GTG CTG AAG CGG GCT ATC CGC ACC CTT TGG ACC ATC CTG GAT GTT ACG GAC
 Y   T   S   V   L   K   R   A   I   R   T   L   W   T   I   L   D   V   T   D

                                                                   302
CAA ATG TGG GTG CCC GTG GTG CGC ACC TGG CGC CTC AAT GAG CGG CAC TAT GGA GGT CTC
 Q   M   W   V   P   V   V   R   T   W   R   L   N   E   R   A   Y   G   G   L

                                                                   362
ACG GGC CTC AAT AAG GCT GAG ACG GCT GCA AAG CAT GGG GAG GAG CAG GTG AAG ATC TGG
 T   G   L   N   K   A   E   T   A   A   K   H   G   E   E   Q   V   K   I   W

                                                                   422
AGG CGT TCC TTT GAC ACC CCG CCA CCA CCT ATG GAC GAG AAA CAC AAC TAC TAC GCC TCC
 R   R   S   F   D   T   P   P   P   P   M   D   E   K   H   N   Y   Y   A   S

                                                                   482
ATC AGC AAG GAT CGG CGC TAT GCA GGC TTG AAG CCT GAG GAG CTG CCT ACC TGT GAG AGC
 I   S   K   D   R   R   Y   A   G   L   K   P   E   E   L   P   T   C   E   S

                                                                   542
CTC AAG GAC ACC ATT GCC CGG GCT CTG CCC TTC TGG AAT GAG GAG ATC GCA CCT AAG ATT
 L   K   D   T   I   A   R   A   L   P   F   W   N   E   E   I   A   P   K   I

                                                                   602
AAG GCT GGC AAG AGA GTC CTT ATT GCA GCC CAT GGG AAC AGC CTT CGA GGC ATT GTC AAG
 K   A   G   K   R   V   L   I   A   A   H   G   N   S   L   R   G   I   V   K

                                                                   662
CAT CTG GAA GGG ATG TCA GAC CAG GCC ATC ATG GAA CTG AAT CTG CCC ACC GGA ATC CCC
 H   L   E   G   M   S   D   Q   A   I   M   E   L   N   L   P   T   G   I   P

                                                                   722
ATT GTC TAT GAG CTG AAC CAG GAG CTG AAG CCC ACC AAG CCC ATG AGG TTC CTG GGA GAC
 I   V   Y   E   L   N   Q   E   L   K   P   T   K   P   M   R   F   L   G   D

                                                                   782
GAA GAG ACA GTT CGG AAG GCC ATG GAG GCT GTG GCT GCC CAG GGA AAG GCA AAG TGA gag
 E   E   T   V   R   K   A   M   E   A   V   A   A   Q   G   K   A   K   *

tgggtgggcggaattc
```

Figure 3
Nucleotide sequence of the coding region of rat PGAM-M cDNA and the deduced protein sequence.

cDNA. Human and rat cDNA also possess the same consensus sequence of the initiator site: CCACCATGG (18). The G+C content of both cDNA is also similar: 57% in rat and 62% in human, and the frequency of G+C present in the third position of codons is

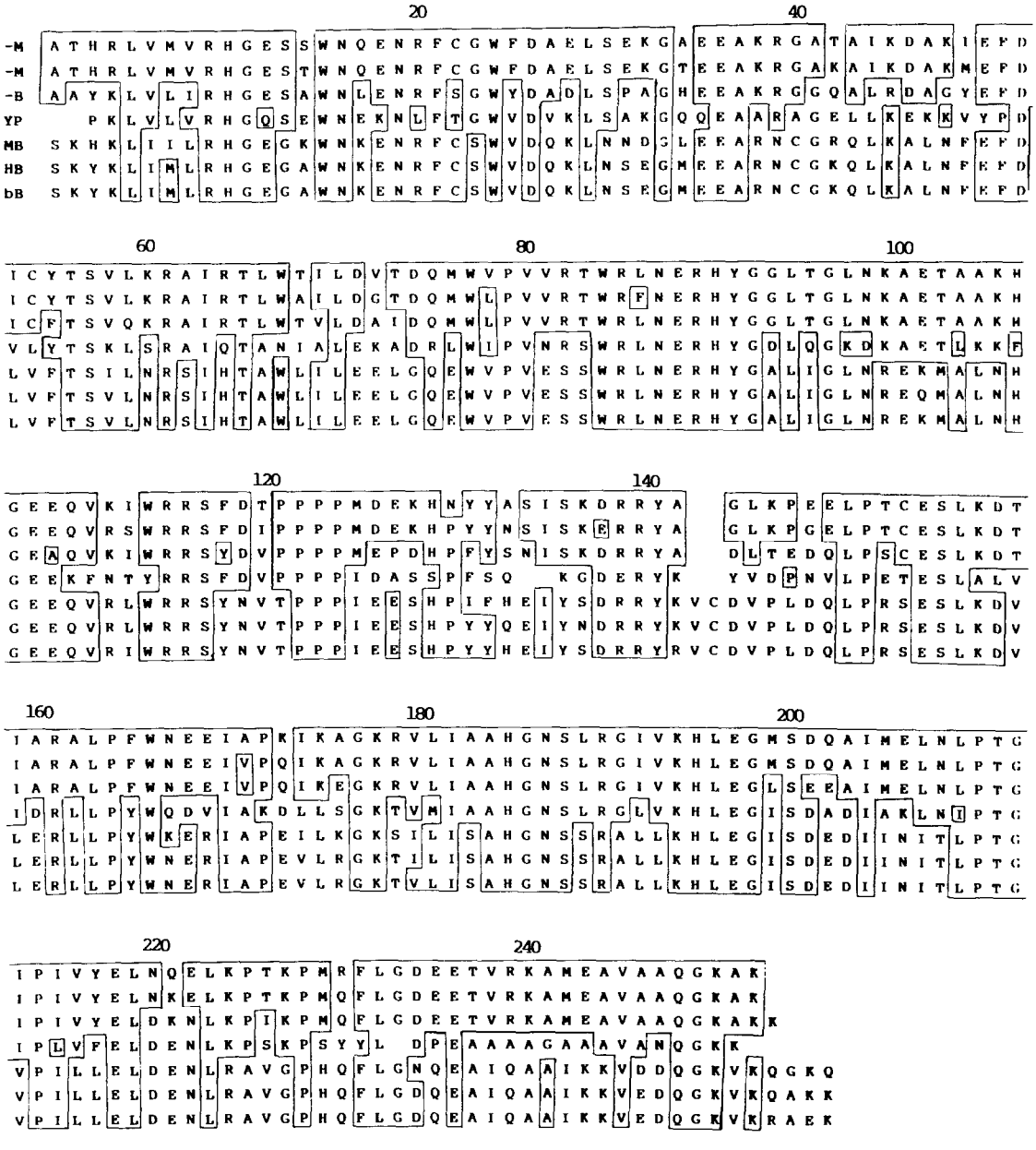

Figure 4
Alignment of the deduced amino acid sequences of rat PGAM-M (RP-M), human PGAM-M (HP-M), Human PGAM-B (HP-B), yeast PGAM (YP), murine BPGM (MB), human BPGM (HB) and rabbit BPGM (RbB). Gaps were introduced to maximize the putative homologies. The amino acid residues largely conserved are boxed within solid lines.

somewhat lower, 73% for rat and 83% for human cDNA, but both values are in agreement with that of Newgard et al. (19) who describe a high third positon G+C value in skeletal muscle.

Both rat and human proteins are perfectly colinear, with an identical length of 252 amino acids (plus the initiator Met). This result and those resulting from comparison of rat

cDNA with human muscle cDNA (3) and human gene (6), strongly suggests that the coding region is complete. It also indicates that translation of the mRNA begins with methionine in the location corresponding to the initiator ATG codon in the cDNA sequences of human PGAM (3). Amino acid alignment with human PGAM (shown in figure 4) suggests that the initiator methionine is released from the protein by post-translational modifications and that the Ala constitutes the first amino acid in the mature protein. This amino terminus is typical of mammalian PGAMs but not of yeast PGAM or BPGMs (1).

Comparison of the deduced amino acid sequences of PGAM-M from rat and human muscle shows 16 substitutions, but none are located in the amino acids supposedly belonging to the active site. Thus, His-10, His-185, Arg-9, Arg-61 and Glu 88 are conserved. Cys-22, which could also contribute to ligand binding and is responsible for the sensitivity to thiol reagents, is also conserved. Only two of the amino acid changes are located in the conservative zones in relation to other PGAMs and BPGMs (Pro-130 and Gln-230), but two other (Glu-138 and Val-171) increased the homology among them.

It has been described that the last 14 residues from the C-terminus constitute a flexible tail particularly susceptible to proteolysis in yeast and in muscle enzymes and when removed, the enzyme losses its activity (20-22). In yeast PGAM, it has been suggested that the C-terminal tail adopts a conformation that could modulate access to the active site (21). Human PGAM-M and PGAM-B have identical C-terminal sequence but it is only 50% homologous with respect to the yeast enzyme. As described by Shanske et al., (3), and since this tail has no presumed catalytic function, these alterations with respect to yeast enzyme do not affect its function or the subunit association.

The secondary structure predicted for rat PGAM-M by the conformational parameters of each amino acid has also been studied by the Microgenie programme (Beckman Corp.). The four parameters analyzed, alpha and beta structures, charge distribution and hydropathy profile, are practically the same as for human PGAM-M, which suggests that the amino acid changes observed in rat PGAM-M sequence are not involved in the maintenance of the native conformation and that all the PGAM family possesses similar secondary and tertiary structures.

ACKNOWLEDGMENTS

This work was supported from grants from the CAYCIT (PB85-0020), PLANICYT (BIO88-0282) and FIS (87-1253). J. Castellà and L. Montoliu are recipients of a research fellowship from M.E.C.

REFERENCES

1- Fothergill-Gilmore, L.A. and Watson, H.C. (1989) In Advances in Enzymology (A. Meister, Ed.) Vol 62, pp. 227-313. John Willey & Sons, New York
2- Bartrons, R. and Carreras, J. (1982) Biochim. Biophys. Acta 708, 167-177
3- Shanske, S.; Sakoda, S.; Hermodson, M.A.; DiMauro, S. and Schon, E.A. (1987) J. Biol. Chem. 262, 14612-14617
4- Sakoda, S.; Shanske, S.; DiMauro, S. and Schon, E.A. (1988) J. Biol. Chem. 263, 16899- 16905
5- Castellà, J.; Ureña, J.; Ludevid, D.; Carreras, J. and Climent, F. (1988) Biochim. Biophys. Acta 956, 97-102
6- Castellà-Escolà, J.; Ojcius, D.M.; Le Boulch, P.; Joulin, V.; Blouquit, Y.; Garel, M.C.; Valentin, C.; Rosa, R.; Climent-Romeo, F. and Cohen-Solal, M. Gene (In Press)
7- Chiba, H. and Sasaki, R. (1978) In Current Topics in Cellular Regulation (B.L. Horecker and R.R. Stadtman, Eds.) Vol 14, pp. 75-116, Academic Press, New York
8- Rose, Z.B. (1980) In Advances in Enzymology (A. Meister, Ed.) Vol 51, pp. 211-253. John Willey & Sons, New York
9- Pons, G.; Bartrons, R. and Carreras, J. (1985) Biochem. Biophys. Res. Commun. 129, 658-663
10- Joulin, V.; Peduzzi, J.; Roméo, P.H.; Rosa, R.; Valentin, C.; Dubart, A.; Lapeyre, B.; Blouquit, Y.; Garel, M.C.; Goossens, M.; Rosa, J. and Cohen-Solal, M. (1986) EMBO J. 5, 2275-2283
11- Yanagawa, S.; Hitomi, K.; Sasaki, R. and Chiba, H. (1986) Gene 44, 185-191
12- Le Boulch, P.; Joulin, V.; Garel, M.C.; Rosa, J. and Cohen-Solal, M. (1988) Biochem. Biophys. Res. Commun. 156, 874-881
13- White, M.F. and Fothergill-Gilmore, L.A. (1988) FEBS Lett. 229, 383-387
14- Graña, X.; Ureña, J.; Ludevid, D.; Carreras, J. and Climent, F. Eur. J. Biochem. (In Press)
15- Andrés, V.; Cussó, R. and Carreras, J. (1989) Differentiation 41, 72-77
16- Davis, R.W.; Bolstein, D. and Roth, R.J. (1980) In A Manual for Genetic Engineering. Advanced Bacterial Genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor. New York
17- Sanger, F.; Nicklen, S. and Coulsen, A.R. (1971) Proc. Natl. Acad. Sci. U.S.A. 74, 5463-5467
18- Kosak, M. (1984) Nucleic Acids Res. 12, 857-872
19- Newgard, C.B.; Nakano, K.; Hwang,, P.K. and Fletterich, R.J. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 8132-8136
20- Sasaki, R.; Sugimoto, E. and Chiba, H. (1966) Arch. Biochem. Biophys. 115, 53-61
21- Winn, S.I.; Watson, H.C.; Harkins, R.N. and Fothergill, L.A. (1981) Phil. Trans. R. Soc. London B 293, 121-130
22- Price, N.C.; Duncan, D. and McAlister, J.W. (1985) Biochem. J. 229, 167-171