
Nucleotide sequence of cDNA encoding the catalytic subunit of phosphorylase kinase from rat soleus muscle

Kendra C.Cawley, Chidambaram Ramachandran, Fredric A.Gorin and Donal A.Walsh

Department of Biological Chemistry and Neurology, School of Medicine, University of California, Davis, CA 95616, USA

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Phosphorylase kinase is a key enzyme of glycogenolysis, with a hexadecameric protein structure of $\alpha_4\beta_4\gamma_4\delta_4$. The γ subunit is catalytic and the others are regulatory. Two isozymes exist, w and r, distinguished by the size of their α subunits; the other subunits are of apparently equal size in the two isozymes. Isozyme distribution in skeletal muscle is correlated with metabolic energy source. The w isozyme is the predominant form due to the high proportion of FG and FOG muscle fiber types. Soleus muscle is distinctive, being composed almost entirely of SO fibers containing the r isozyme (1).

We have cloned a cDNA which contains the complete coding region for the γ subunit of phosphorylase kinase from a λ gt10 library prepared from adult rat soleus muscle (obtained from Dr.F.Schachat, Department of Anatomy, Duke University). The library was screened with a 75 bp oligonucleotide probe, the sequence of which was based on the amino acid sequence of the rabbit γ subunit (2) employing empirically derived codon usage rules (3). The probe was directed against a region believed to be unique to phosphorylase kinase and not shared by other kinases. The probe proved to be 87% homologous with the corresponding nucleotide sequence in the cDNA (underlined).

Amino acid sequences for the γ subunit derived from mouse and rabbit cDNAs have been recently reported (4-6). The mouse sequences shared 93% sequence identity with the amino acid sequence determined directly for the γ -subunit of the w isozyme from rabbit skeletal muscle. The amino acid homology between the sequence presented here and that of the mouse γ -subunit is 99%. At the nucleotide level, the homology within the coding region is 94% compared with the mouse cDNAs, and of the 68 nucleotide differences, 61 are in the third position of the codon. The high degree of homology between the sequence presented here, derived from rat soleus muscle cDNA and the rabbit and mouse sequences described for the w isozyme suggests that the γ subunit in the r and w isozymes may be identical. (This work was supported by NIH Grant # DK13613)

5' GAATTCCTGTCACTTCCATTCAAGAGCCTACAAGAACCTAACTAGGTGCTTGGGATCCCCTGAGATCCAGCTGACCATG

80 ACCCGGGATGAGGCCCTCCCTGACTCTCATTCTGCACAGAATTTCTATGAGAAGCTACGAGCCCAAGGAGATCCTGGGCAGGGAGTCAGC
T R D E A L P D S H S A Q N F Y E N Y E P K E I L G R G V S 30170 AGCGTGGTCAGGAGATGCATTACAAACCTACGTGCCAAGAATACGCAGTAAAAATCATCGACATCACCGCGGAGGAAGCTTTAGCTCC
S V V R R C I H K P T C Q E Y A V K I I D I T G G G S F S S 60260 GAGGAGGTACAGGAGCTTCGGGAAGCCACCCTGAAGGAGGTGGACATTTCTGAGAAGGTCTCAGGACACCCCAACATCATACAGCTGAAG
E E V Q E L R E A T L K E V D I L Q K V S G H P N I I Q L K 90350 GACACTTACGAGACCAACACTTCTCTCTCTGGTATTGTATCTGATGAAGAGAGGGGAAGCTTTGACTATCTCACAGAGAAGGTCAAC
D T Y E T N T F F F L V F D L M K R G E L F D Y L T E K V T 120440 TTGACTGAGAAGAAACCGAAGATCATCGCGCCCTGCTGGAGTGGTCTGCACCTTGACAACTCAACATTGTCCATCGGGACCTG
L T E K E T R K I M R A L L E V V C T L H K L N I V H R D L 150530 AAGCCGGAGAACATCCTTCTGGATGACAACATGAACATCAAGCTCACTGACTTCGGGTTTTCTGCCAGCTGCAGCCAGGAGAGAAGCTC
K P E N I L L D D N M N I K L T D F G F S C Q L Q P G E K L 180620 CGAGAGGTTTGTGGGACTCCCAGTTATCTGCCCCCGAAATCATCCAGTGCTCCATGGACGAAGGCCATCCTGGCTATGGAAAGGAGGTG
R E V C G T P S Y L A P E I I Q C S M D E G H P G Y G K E V 210

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710 GACATGTGGAGCACAGGAGTCATCATGTATACGCTGCTGGCTGGCTCCCCGCCCTTCTGGCACCGGAAGCAAATGCTAATGTTGAGGATG
D M W S T G V I M Y T L L A G S P P F W H R K Q M L M L R M 240

800 ATCATGGATGGCAAATACCACTTCGGCTCACCAGAGTGGGATGACTACTCAGATACCGTGAAAGACTTGGTATCTCGCTTCTTGGTGGTG
I M D G K Y Q F G S P E W D D Y S D T V K D L V S R F L V V 270

890 CAACCCAGGACCGCTGCTCGGCGGAAGAGGCCCTGGCGCATCCTTTCTCCAGGAGTACGTGGTGAAGAGGTGCGGCACCTCAGCCCT
Q P Q D R C S A E E A L A H P F F Q E Y V V E E V R H F S P 300

980 CGAGGGAAGTTCAAGGTCACTGTCTAACTGTGCTGGCCTCGGTAAGGATCTACTACCAGTACCGTCGGGTGAAGCCGGTAACCAAGGGAG
R G K F K V I C L T V L A S V R I Y Y Q Y R R V K P V T R E 330

1070 ATCGTCATCCGAGACCCCTACGCCCTACGCCATTGCGCAGACTCATCGACGCCTATGCTTCCGCATCTACGGCCACTGGTGAAGAAA
I V I R D P Y A L R P L R R L I D A Y A F R I Y G H W V K K 360

1160 GGGCAACAGCAGAACAGAGCTGCCCTCTTCGAGAACACGCCCAAGGCAGTGTCTCTCTCCTTGGCTGAGGAGGAGGACTTCTGAGGGGTG
G Q Q Q N R A A L F E N T P K A V L L S L A E E E D F

1250 GCGGGGCACAGGGGAGAAAGCAAACAGGGTACGCGCCCCAGAAGTGTGAGAAGAGGATGCCTCAAGGAGGAGGCTTCTGCAAAGGAGCT

1340 GTGACCCTACTCTGCACTCTCAAAGGCTCGCCACTAGATGCGGGAATTC 3'

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