Amino acid sequence of a novel protein phosphatase 1 binding protein (R5) which is related to the liver- and muscle-specific glycogen binding subunits of protein phosphatase 1

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Abstract A full-length cDNA encoding a novel human protein phosphatase 1 (PP1) binding subunit of molecular mass 36 kDa, termed PPP1R5, was sequenced. PPP1R5 shows 42% identity to the glycogen binding subunit (G_L) of PP1 from rat liver and 28% identity to the N-terminal region of the glycogen binding subunit (G_M) of PP1 from human skeletal muscle. Like G_L, PPP1R5 modulates the specificity of PP1, but it differs from G_L in being present in a wide variety of tissues, besides liver. The amino acid sequence and properties of PPP1R5 indicate that it is not subject to the same modes of covalent and allosteric regulation by hormones as are G_M and G_L.

Key words: Protein phosphatase 1; Glycogen metabolism; cDNA; Chromosome 10

1 Introduction

Protein phosphatase 1 (PP1) participates in the regulation of a wide variety of cellular functions by reversible protein phosphorylation and is one of the major protein phosphatases dephosphorylating serine and threonine residues in eukaryotes. The ability of PP1 to regulate diverse functions resides in the capacity of PP1 to interact with a variety of regulatory subunits that may target PP1 to specific subcellular locations, modulate its substrate specificity and allow its activity to be responsive to extracellular signals [1].

A 126 kDa glycogen binding subunit (G_M, PPP1R3) targets PP1 to glycogen particles and to the sarcoplasmic reticulum in striated muscle [2,3]. The hormones insulin and adrenalin are thought to influence the activity of PP1 via the G_M subunit. Phosphorylation of Ser-46 in human G_M, in response to insulin, enhances the rate at which PP1 dephosphorylates and activates glycogen synthase causing an increase in glycogen synthesis [4]. In contrast, phosphorylation of Ser-65 in human G_M by protein kinase A in response to β-adrenergic agonists triggers dissociation of PP1 from G_M, thus inhibiting PP1 from acting on glycogen synthase and phosphorylase and resulting in decreased glycogen synthesis and stimulation of glycogenolysis [5].

A distinct 33 kDa glycogen binding subunit (G_L, PPP1R4), which is only 23% identical to the N-terminal portion of G_M, targets PP1 to glycogen in liver [6,7]. The binding of G_L modulates the activity of PP1, enhancing the rate at which it dephosphorylates and activates glycogen synthase and suppressing the rate at which it inactivates phosphorylase. Hormonal regulation of the activity of PP1 in liver is not known to occur through the phosphorylation of G_L. Instead, the hormone glucagon (acting via cyclic AMP and protein kinase A) and α-adrenergic agonists (acting via Ca^{2+}) increase the levels of phosphorylase *a*, which to binds G_L and potently inhibits PP1 at nanomolar concentrations. This inhibition is thought to be allosteric, since the K_m for phosphorylase as a substrate of PP1 is in the micromolar range. Insulin acts by lowering the level of cyclic AMP in liver, thereby decreasing the level of phosphorylase *a* and relieving the inhibition of the PP1 G_L complex. Glycogen synthesis in liver is also stimulated by glucose, which binds to phosphorylase *a* increasing the rate at which it is dephosphorylated.

Several other targetting subunits of PP1 have now been identified in mammals. These include the myosin binding targetting complex (comprising an M_{110} and an M_{21} subunit) of smooth muscle, which enhances the dephosphorylation of myosin light chains by PP1 and is involved in the relaxation of smooth muscle [8,9]. A distinct myosin targetting subunit of PP1 is present in striated muscles [10]. A p53 binding protein (53BP2) [11], a nuclear protein NIPP-1 [12] and an RNA splicing factor PSF1 [13] have been shown to bind to PP1. The retinoblastoma gene product [14], ribosomal proteins L5 [15] and RIPP-1 [16] and a 110 kDa nuclear protein yet to be identified [17] are also reported to interact with PP1. The small cytosolic proteins, inhibitor-1, inhibitor-2 and DARPP-32, inhibit PP1 (reviewed in [18]). A complex between inhibitor-2 and PP1 has been isolated. More recently, inhibitor-2 has been shown to act like a molecular chaperone to fold PP1 into its native conformation [19,20]. A number of distinct PP1 targetting subunits have also been identified in yeast (reviewed in [21]).

Sites on the glycogen and myofibrillar targetting subunits which bind to PP1 have been localised [22] and a 13 residue peptide containing a small motif common to many of the PP1 binding subunits has been crystallised as a complex with PP1 [23]. Here we report a novel PP1 binding subunit, which we term PPP1R5 in accordance with the human genome nomenclature [24], which contains the PP1 binding motif, modulates the specificity of PP1 and is related to G_L but has a much wider tissue distribution.

2. Materials and methods

2.1. Identification and sequence analysis of the cDNA encoding human protein phosphatase 1 binding subunit R5

The Human Genome Sciences expressed sequence (EST) cDNA database, derived from over 500 human cDNA libraries [25,26], was searched for sequences related the rat liver glycogen binding subunit (G_L) using the TBLASTN algorithm [27]. Four overlapping cDNA sequences, identified from different libraries, encoded part of a protein

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with sequences similarities to rat G_L, which we termed PPP1R5. One of these cDNAs, pHGBDX21, which encoded PPP1R5 and derived from a human gall bladder library, was completely sequenced in both directions on an Applied Biosystems 373A automated DNA sequencer using Taq dye terminator cycle sequencing. A liver cDNA kindly provided by the I.M.A.G.E. consortium, St. Louis, was also shown by sequence analysis to encode part of PPP1R5. The complete sequence of PPP1R5 was searched against a more recent update of the EST library organised into overlapping assemblies, and this identified 58 further ESTs which spanned the entire coding region and an additional 1379 nucleotides of the 3′ untranslated region (UTR) not included in the original cDNA. Hence the entire mRNA would appear to be at least 2.5 kb. The extreme 3′ sequence also overlapped two sequence tagged site sequences (STS identifiers: WI-11129 and TIGR-A004S47) which have been localised to chromosome 10q23–24 (Whitehead/MIT Center).

2.2. Expression of GST-R5 fusion protein in E. coli and production of antibodies

The open reading of PPP1R5 was amplified by PCR using oligonucleotide 5′-GCGCCATATGAGCTGCACCAGAATGATC-3′, which creates an *Nde*I site (underlined) at the initiating methionine codon, and oligonucleotide 5′-GCGCCTCGAGTCATCGATAA-GAGGCCAAGTTC-3′, which creates an *Xho*I site (underlined) just 3′ of the termination codon. The complete coding region of PPP1R5 was cloned into the prokaryote gene fusion expression vector pGEX-AH [7,28]. The final construct termed, pGEX-PPP1R5, encoded glutathione *S*-transferase (GST) followed by the complete open reading frame of PPP1R5. The GST-R5 protein was expressed in *E. coli* and purified by affinity chromatography on glutathione-agarose (Sigma) as described in [7]. 250 μg of soluble GST-R5 was isolated per litre of bacterial culture. Antibodies to GST-R5 were raised in sheep and affinity purified. For measurement of the effect of PPP1R5 on the PP1 activity, contaminating GST in the GST-R5 preparation was removed by chromatography on Superdex 200 in 50 mM Tris-HCl pH 7.5, 0.03% Brij 35, 0.1% 2-mercaptoethanol. GST-R5 eluted as a dimer, probably because GST possesses a dimeric structure.

2.3. Glutathione affinity precipitations

Indicated amounts of GST-R5 and PP1 were incubated in 50 or 100 μl of 50 mM Tris-HCl pH 7.5, containing 150 mM NaCl, 0.1% (v/v) 2-mercaptoethanol, 0.02% (v/v) Brij 35 and 0.1 mg/ml bovine serum albumin for 1 h at 4°C. 10 μl of glutathione-agarose (Sigma) was added and incubation was continued at 4°C with shaking for 30 min. After centrifugation, the supernatant was removed and the pellet washed twice with 1 ml buffer (without albumin), then denatured by heating in SDS gel loading buffer at 95°C for 5 min and analysed by polyacrylamide gel electrophoresis.

2.4. Protein phosphatase assays

PP1 was human PP1γ_1 [29] expressed in *E. coli* and purified as described in [19]. Rabbit skeletal muscle glycogen phosphorylase (prepared by Miss F. Douglas) was ^{32}P-labelled by phosphorylase kinase to a stoichiometry of 1 mol phosphate per mol subunit as in [30] and was 10 μM in the assays. Protein phosphatase assays were performed in the absence of divalent cations as described in [30]. One unit of activity is the amount of enzyme which catalyses the release of 1 μmol of [^{32}P]phosphate per minute.

3. Results

3.1. Human protein phosphatase 1 binding subunit R5 is related to, but not the homologue of, rat liver glycogen binding subunit of PP1

A search of the Human Genome Sciences EST database with the sequence of the glycogen binding subunit (G_L) of

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                                    c acgaggccgcgggggcaaggcctggagctg  -61
tggttcgaatttgtgcaggcagcgggtgct ggcttttagggtccgccgcctctctgcctа   -1
ATGAGCTGCACCAGAATGATCCAGGTTTTA GATCCACGTCCTTTGACAAGTTCGGTCATG   60
 M  S  C  T  R  M  I  Q  V  L   D  P  R  P  L  T  S  S  V  M   20

CCCGTGGATGTGGCCATGAGGCTTTGCTTG GCACATTCACCACCTGTGAAGAGTTTCCTG  120
 P  V  D  V  A  M  R  L  C  L   A  H  S  P  P  V  K  S  F  L   40

GGCCCGTACGATGAATTTCAACGACGACAT TTTGTGAATAAATTAAAGCCCCTGAAATCA  180
 G  P  Y  D  E  F  Q  R  R  H   F  V  N  K  L  K  P  L  K  S   60

TGTCTCAATATAAAACACAAAGCCAAATCA CAGAATGACTGGAAGTGCTCACACAACCAA  240
 C  L  N  I  K  H  K  A  K  S   Q  N  D  W  K  C  S  H  N  Q   80

GCCAAGAAGCGCGTTGTGTTTGCTGACTCC AAGGGCCTCTCTCTCACTGCGATCCATGTC  300
 A  K  K  R  V  V  F  A  D  S   K  G  L  S  L  T  A  I  H  V  100

TTCTCCGACCTCCCAGAAGAACCAGCGTGG GATCTGCAGTTTGATCTCTTGGACCTTAAT  360
 F  S  D  L  P  E  E  P  A  W   D  L  Q  F  D  L  L  D  L  N  120

GATATCTCCTCTGCCTTAAAACACCACGAG GAGAAAAACTTGATTTTAGATTTCCCTCAA  420
 D  I  S  S  A  L  K  H  H  E   E  K  N  L  I  L  D  F  P  Q  140

CCTTCAACCGATTACTTAAGTTTCCGGAGC CACTTTCAGAAGAACTTTGTCTGTCTGGAG  480
 P  S  T  D  Y  L  S  F  R  S   H  F  Q  K  N  F  V  C  L  E  160

AACTGCTCATTGCAAGAGCGAACAGTGACA GGGACTGTTAAAGTCAAAAATGTGAGTTTT  540
 N  C  S  L  Q  E  R  T  V  T   G  T  V  K  V  K  N  V  S  F  180

GAGAAGAAAGTTCAGATCCGTATCACTTTC GATTCTTGGAAAAACTACACTGACGTAGAC  600
 E  K  K  V  Q  I  R  I  T  F   D  S  W  K  N  Y  T  D  V  D  200

TGTGTCTATATGAAAAATGTGTATGGTGGC ACAGATAGTGATACCTTCTCATTTGCCATT  660
 C  V  Y  M  K  N  V  Y  G  G   T  D  S  D  T  F  S  F  A  I  220

GACTTACCCCCTGTCATTCCAACTGAGCAG AAAATTGAGTTCTGCATTTCTTACCATGCT  720
 D  L  P  P  V  I  P  T  E  Q   K  I  E  F  C  I  S  Y  H  A  240

AATGGGCAAGTCTTTTGGGACAACAATGAT GGTCAGAATTATAGAATTGTTCATGTTCAA  780
 N  G  Q  V  F  W  D  N  N  D   G  Q  N  Y  R  I  V  H  V  Q  260

TGGAAGCCTGATGGGGTGCAGACACAGATG GCACCCCAGGACTGTGCATTCCACCAGACG  840
 W  K  P  D  G  V  Q  T  Q  M   A  P  Q  D  C  A  F  H  Q  T  280

TCTCCTAAGACAGAGTTAGAGTCAACAATC TTTGGCAGTCCGAGGCTGGCTAGTGGGCTC  900
 S  P  K  T  E  L  E  S  T  I   F  G  S  P  R  L  A  S  G  L  300

TTCCCAGAGTGGCAGAGCTGGGGGAGAATG GAGAACTTGGCCTCTTATCGATGAattaag  960
 F  P  E  W  Q  S  W  G  R  M   E  N  L  A  S  Y  R             317

caacaatgtaactggtcttgacttgtcata ttcccccatgcaatcctaggtctgtattgc 1020
tcaattttaggaagcctttgctactccatc agtaggtttagatttga               1067
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Fig. 1. Complementary DNA and predicted protein sequence of the human protein phosphatase 1 binding subunit R5 (PPP1R5).

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R5    MSCTRMIQVL DPRPLTSSVM PVDVAMRLCL AHSPPVKSFL GPYDEFQRRH  50
                            M AVDIEY---- -------SYS SMAPSLRRER  20
G_L

R5    FVNKL----- KPLKSCLNI- -KHKAKSQND WKCSHNQAKK KVVFADSKGL  93
                 FTFKISPKLN KPLRPCIQLG SKDEAGRMVA PTVQEKKVKK KVSFADNQGL  70
G_L

R5    SLTAIHVFSD LPEEPAWDLQ FDLLDLNDIS SALKHHEEKN LILDFPQPST 143
                 ALTMVKVFSE F--DDPLDIP FNITELLDNI VSLTTAESES FVLDFPQPSA 118
G_L

R5    DYLSFRSHFQ KNFVCLENCS LQERTVTGTV KVKNVSFEKK VQIRITFDSW 193
                 DYLDFRNRLQ TNHVCLENCV LKEKAIAGTV KVQNLAFEKV VKIRMTFDTW 168
G_L

R5    KNYTDVDCVY MKNVYGGTDS DTFSFAIDLP PVIPTEQKIE FCISYHANGQ 243
                 KSFTDFPCQY VKDTYAGSDR DTFSFDISLP EKIQSYERME FAVCYECNGQ 218
G_L

R5    VFWDNNDGQN YRIVHVQWKP DGVQTQMAPQ DCAFHQTSPK TELESTIFGS 293
                 SYWDSNKGKN YRITRAELRS TQGMTE---- ----PYNGPD FGISFDQFGS 260
G_L

R5    PRLASGLFPE WQSWGRMENL ASYR                              317
                 PRCSFGLFPE WPSYLGYEKL GPYY                      284
G_L
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Fig. 2. Comparison of the amino acid sequences of human protein phosphatase 1 binding subunit R5 with the glycogen binding subunit (G_L) of protein phosphatase 1 from rat liver. Identities are shown by vertical lines. Conservative amino acid changes are indicated by colons in the following groups (L, I, V); (A, G); (S, T); (D, E); (Q, N); (R, K); (F, Y, W). Residues that are identical in R5, G_L and PPP1R3 (G_M) are underlined. The minimal PP1 binding motif is double underlined.

PP1 from rat liver identified a number of partially sequenced cDNAs encoding a protein with significant sequence similarity to rat G_L. The complete sequence of the open reading frame encoded by one of these cDNAs from human gall bladder is presented in Fig. 1. The predicted protein is 36.4 kDa, comprising 317 amino acids if the initiating methionine is at codon 1 rather than at codon 6, 20 or 26. An in-frame termination codon precedes codon 1. However, nucleotide sequences immediately preceding codons 6, 20 or 26 conform more closely to the consensus sequence for eukaryote translation initiation [31] than that preceding codon 1. Translation may therefore start at codon 6 giving rise to a protein of 35.8 kDa.

The amino acid sequence of human PPP1R5 shows 42% identity to rat liver G_L and 51% similarity if conservative substitutions are taken into account (Fig. 2). PPP1R5 is less similar to G_M showing only 27% and 28% identity to the first 235 amino acids of rabbit and human G_M respectively. The low degree of similarity of PPP1R5 to G_L and G_M indicates that PPP1R5 is unlikely to be the human homologue of rat G_L. The glycogen binding subunits from human and rabbit skeletal muscle show a much higher level of identity (73%) to each other [3]. The different tissue distributions of human PPP1R5 and rat G_L also indicate that these two proteins are not species homologues. PPP1R5 cDNA was identified in cDNA libraries from several adult tissues and cells, including gall bladder, prostate, osteoblasts, retina, smooth muscle, liver, kidney medulla, striatum and senescent fibroblasts, several fetal tissues including brain, lung, liver, heart, spleen and placenta, and tumour cells such as osteosarcoma, hepatocellular, ovarian and melanocyte tumour cells. In contrast, G_L appears to be a liver-specific protein since its mRNA was present in liver but undetectable in lung, brain, heart, spleen, skeletal muscle, kidney and testis [7]. Affinity purified antibodies to GST-PPP1R5 recognised a 36 kDa protein on an immunoblot of rat liver and skeletal muscle extracts, whereas G_L (33 kDa) was only detected in liver. A subsequent search of an assembled version of the EST database revealed an additional 1.38 kb of 3′ UTR suggesting an overall mRNA size of more than 2.5 kb. The 3′ UTR overlapped the sequences of two

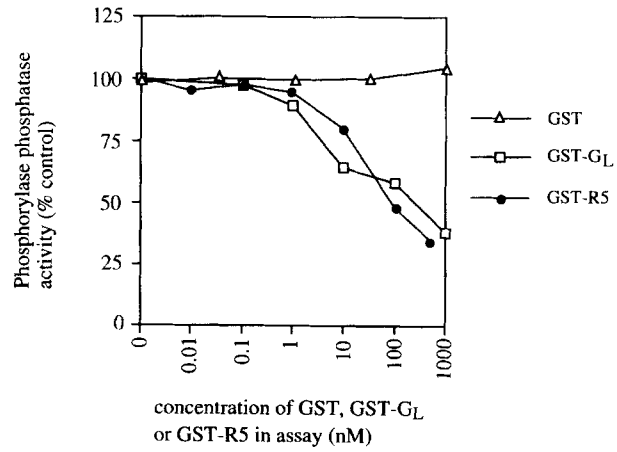

Fig. 3. Comparison of the effects of the GST-R5 and GST-G_L on the phosphorylase phosphatase activity of protein phosphatase 1. The catalytic subunit (PP1γ) was diluted in assay buffer to 1.5 U/ml (1.4 nM), and a 0.01 ml aliquot was incubated for 15 min at 30°C with 0.01 ml of the indicated concentrations of the purified bacterially expressed GST-R5, GST-G_L or GST diluted in the same buffer. Assays were initiated with 0.01 ml ^{32}P-labelled phosphorylase. The phosphorylase phosphatase activities of PP1 in the presence of GST-R5 (open squares) and GST-G_L (filled circles) and GST (open triangles) are shown. Activities are presented as a percentage of the initial PP1 activity.

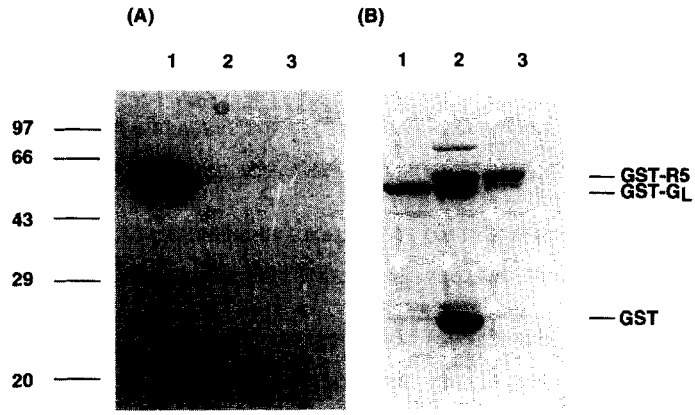

Fig. 4. Comparison of the binding of phosphorylase *a* to GST-G_L and GST-R5. GST-R5 and GST-G_L were electrophoresed on a 10% polyacrylamide gel, transferred to nitrocellulose membranes and probed with (A) ^{32}P-labelled phosphorylase *a* as described in [6,7] or (B) stained with Coomassie blue. The positions of GST-R5, GST-G_L and GST are indicated. Lane 1, affinity purified GST-G_L; lane 2, affinity purified GST-R5; lane 3, GST-R5 after gel filtration on Superdex 200. The molecular mass markers are phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa) and soybean trypsin inhibitor (20 kDa).

sequence tagged sites, which allows the gene for PPP1R5 to be localised to chromosome 10q23–24.

3.2. *Human PPP1R5 coprecipitates with PP1 and modulates the substrate specificity of PP1*

In order to determine whether PPP1R5 would bind to PP1 we expressed R5 in *E. coli* as a GST fusion protein (Fig. 4), incubated the purified GST-R5 with bacterially expressed PP1 and performed precipitation studies using glutathione-agarose beads as described in Section 2.3. SDS polyacrylamide gel analysis of the pellets showed that PP1 was precipitated in the presence of GST-R5 and GSH-agarose beads but not in control incubations containing only PP1 and GSH agarose (data not shown).

Affinity purified GST-R5 inhibited the phosphorylase phosphatase activity of PP1 as observed previously for GST-G_L [7]. The amount of inhibition varied with the different preparations of GST-R5 and GST-G_L, probably due to the aggregation of both protein preparations (see Section 4). Fig. 3 shows that inhibition observed with freshly prepared preparations of GST-R5 and GST-G_L was similar with an IC_{50} of approximately 100 nM. Removal of GST, which was a major contaminant of the PPP1R5 preparation, did not significantly affect the IC_{50}.

The glycogen synthase phosphatase activity of the complex of PP1 with G_L is inhibited by phosphorylase *a*, the inhibition being achieved by the binding of phosphorylase *a* to the G_L subunit [7]. PPP1R5 was therefore tested for its ability to bind phosphorylase *a*. Fig. 4 shows that phosphorylase *a* bound to GST-G_L but it did not bind to GST-R5, indicating that R5 is distinct from G_L in this property and is not regulated by phosphorylase *a*.

4. Discussion

The human protein PPP1R5 identified here is most closely related to the rat liver glycogen binding subunit (G_L) of PP1, but the fairly low sequence identity to G_L and much wider tissue distribution indicate that it is unlikely to be the human homologue of G_L. PPP1R5 also differs from G_L in that it does not bind phosphorylase *a*, which is known to regulate the activity of the PP1G_L complex. Coprecipitation studies dem-

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R5    157  VCLE---NCSLQERTVTGTVKVKNVSFEKKVQIRI--TFDSWKNYT  197
GL    132  VCLE---NCVLKEKAIAGTVKVQNLAFEKVVKIRM--TFDTWKSFT  172
GM    128  AILES-TESLLGSTSIKGIIRVLNVSFEKLVYVRM--SLDDWQTHY  170
GAC1  244  VKLHSLTQLGDDSSKITGLVYVKNLSFEKYLEIKF--TFNSWRDIH  287
AMYL  33   VQLDS---YNYDGSTFSGKIYVKNIAYSKKVTVIYADGSDNWNNNG   75

R5    198  DVDCVYMKNVYGGTDSDTFSFAIDLPPVIPTEQ-------------  230
GL    173  DFPCQYVKDTYAGSDRDTFSFDISLPEKIQSYE-------------  205
GM    171  DILAEYVPNSCDG-ETDQFSFKIVLVPPYQKDGS------------  203
GAC1  288  YVTANFNRTIN--SNVDEFKFTIDLNSLKYILLIKRIITMEKNTSS  331
AMYL  76   NTIAASYSAPISGSNYEYWTFSASINGIK-----------------  104

R5    231  ---KIEFCISYHANGQVFWDNNDGQNYRI                  256
GL    206  ---RMEFAVCYECNGQSYWDSNKGKNYRI                  231
GM    204  ---KVEFCIRYETSVGTFWSNNNGTNYTF                  229
GAC1  332  CPLNIELCCRYDVNNETYYDNNNGKNYHL                  360
AMYL  105  -----EFYIKYEVSGKTYYDNNNSANYQV                  128
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Fig. 5. A putative carbohydrate binding domain identified from sequence similarities between PPP1R5, rat G_L [7], human G_M [3], *S. cerevisiae* GAC1 [33] and *Rhizopus oryzae* glucoamylase (AMYL) [32]. Amino acids that are conserved in all five proteins are underlined and those that are identical are double underlined.

onstrated that PPP1R5 will form a complex with PP1 and inspection of the sequence of PPP1R5 shows that it possesses the RVXF motif (double underlined in Fig. 2), which is involved in the binding of G_M and G_L to PP1 [23] and is common to a number of other PP1 binding proteins [24]. PPP1R5 therefore represents a novel PP1 binding protein.

PPP1R5 and G_L show similar inhibition of phosphorylase phosphatase activity. Therefore like other regulatory subunits of PP1, PPP1R5 may modulate the specificity of PP1 towards particular substrates. The identification of cDNAs encoding PPP1R5 in a large number of different tissues including liver indicates that, unlike G_L which is liver specific, and G_M which is found in muscles (skeletal, diaphragm and heart), PPP1R5 provides a function common to many tissues. At present it is not known whether PPP1R5 binds to glycogen, because the aggregation properties of PPP1R5 (and G_L) cause it to pellet on centrifugation in both the presence and absence of glycogen. However, PPP1R5 does contains a region (amino acids 170–181) homologous to a postulated glycogen binding site in G_M [1]. Interestingly an extensive region of PPP1R5, which does not contain the RVXF PP1 binding site, is conserved in *Rhizopus oryzae* glucoamylase, an enzyme which is secreted from the fungus and degrades starch [32]. An alignment of PPP1R5 (amino acids 157–256), G_L, G_M, *S. cerevisiae* GAC1 [33] and *R. oryzae* glucoamylase (amino acids 33–129) is shown in Fig. 5. Since amino acids 26–109 of *R. oryzae* glucoamylase have been shown to adsorb to raw starch [32], and G_L, G_M and GAC1 all bind to glycogen, it seems likely that part or all of the section shown in Fig. 5 may be a carbohydrate binding domain. *S. cerevisiae* GIP2, a PP1 binding protein whose function is unknown, has also recently been shown to align to part of this region [34]. PPP1R5 may therefore be involved in the regulation of glycogen metabolism in a wide range of tissues, besides liver and muscle. However, the hormonal regulation of PPP1R5 must differ from that of G_M and G_L. Neither of the serine residues that are phosphorylated in G_M in response to insulin and adrenalin are conserved in PPP1R5, nor is the phosphorylase *a* binding site which is involved in the allosteric regulation of G_L by hormones. Further studies will be required to ascertain whether or not PPP1R5 is hormonally regulated.

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References

[1] Hubbard, M.J. and Cohen, P. (1993) Trends Biochem. Sci. 18, 172–177.
[2] Tang, P.M., Bondor, J.A., Swiderek, K.M. and DePoali-Roach, P. (1991) J. Biol. Chem. 266, 15782–15789.
[3] Chen, Y.H., Hansen, L., Chen, M.X., Bjørbæk, C., Vestergaard, H., Hansen, T., Cohen, P.T.W. and Pedersen, O. (1994) Diabetes 43, 1234–1241.
[4] Dent, P., Lavoinne, A., Nakielny, S., Caudwell, F.B., Watt, P. and Cohen, P. (1990) Nature 348, 302–308.
[5] Nakielny, S., Campbell, D.G. and Cohen, P. (1991) Eur. J. Biochem. 199, 713–722.
[6] Moorhead, G., MacKintosh, C., Morrice, N. and Cohen, P. (1995) FEBS Lett. 362, 101–105.
[7] Doherty, M.J., Moorhead, G., Morrice, N., Cohen, P. and Cohen, P.T.W. (1995) FEBS Lett. 375, 284–298.
[8] Alessi, D., Macdougall, L.K., Sola, M.M., Ikebe, M. and Cohen, P. (1992) Eur. J. Biochem. 210, 1023–1035.
[9] Chen, Y.H., Chen, M.X., Alessi, D.R., Campbell, D.G., Shanahan, C., Cohen, P. and Cohen, P.T.W. (1994) FEBS Lett. 356, 51–55.
[10] Dent, P., Macdougall, L.K., Mackintosh, C., Campbell, D.G. and Cohen, P. (1992) Eur. J. Biochem. 210, 1037–1044.
[11] Helps, N.R., Barker, H.M., Elledge, S.J. and Cohen, P.T.W. (1995) FEBS Lett. 377, 295–300.
[12] Van Eynde, A., Wera, S., Beullens, M., Torrekens, S., Van Leuven, F., Stalmans, W. and Bollen, M. (1995) J. Biol. Chem. 270, 28068–28074.
[13] Hirano, K., Erdödi, F., Patton, J.G. and Hartshorne, D.J. (1996) FEBS Lett. 389, 191–194.
[14] Durfee, T., Becherer, K., Chen, P.-L., Yeh, S.-H., Yang, Y., Kilburn, A.E., Lee, W.H. and Elledge, S.J. (1993) Genes Dev. 7, 555–569.
[15] Hirano, K., Ito, M. and Hartshorne, D.J. (1995) J. Biol. Chem. 270, 19786–19790.
[16] Buellens, M., Stalmans, W. and Bollen, M. (1996) Eur. J. Biochem. 239, 183–189.
[17] Jagiello, I., Buellens, M., Stalmans, W. and Bollen, M. (1995) J. Biol. Chem. 270, 17257–17263.
[18] Cohen, P. (1989) Annu. Rev. Biochem. 58, 453–508.
[19] Alessi, D.R., Street, A.J., Cohen, P. and Cohen, P.T.W. (1993) Eur. J. Biochem. 213, 1055–1066.
[20] MacKintosh, C., Garton, A.J., McDonnell, A., Barford, D., Cohen, P.T.W., Cohen, P. and Tonks, N.K. (1996) FEBS Lett. in press.
[21] Stark, M.J.R. (1996) Yeast (in press).
[22] Johnson, D.F., Moorhead, G., Caudwell, F.B., Cohen, P., Chen, Y.H., Chen, M.X. and Cohen, P.T.W. (1996) Eur. J. Biochem. 239, 317–325.
[23] Egloff, M.-P., Johnson, F., Moorhead, G., Cohen, P.T.W., Cohen, P. and Barford, D. (1997) EMBO J. (submitted).
[24] Cohen, P.T.W. (1996) Adv. Prot. Phosphatases 8, 371–376.
[25] Adams M.D., Dubnick, M., Kerlavage, A.R., Moreno, R.F., Kelley, J.M., Utterback, T.R., Nagle, J.W., Fields, C. and Ventner, J.C. (1992) Nature 355, 632–634.
[26] Adams, M.D. et al. (1995) Nature 377, 3–174.
[27] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) J. Mol. Biol. 215, 403–410.
[28] Guan, K.-L. and Dixon, J.E. (1991) Anal. Biochem. 192, 262–267.
[29] Barker, H.M., Craig, S.P., Spurr, N.K. and Cohen, P.T.W. (1993) Biochim. Biophys. Acta 1178, 228–233.
[30] Cohen, P., Alemany, S., Hemmings, B.A., Resink, T.J., Stralfors, P. and Tung, H.Y.L. (1988) Methods Enzymol. 159, 390–408.
[31] Kozak, M. (1991) J. Biol. Chem. 266, 19867–19870.
[32] Tanaka, Y., Ashikari, T., Nakamura, N., Kiuchi, N., Shibano, Y., Amachi, T. and Yoshizumi, H. (1986) Agric. Biol. Chem. 50, 965–969.
[33] François, J.M., Thompson-Jaeger, S., Skroch, J., Zellenka, U., Spevak, W. and Tatchell, K. (1992) EMBO J. 11, 87–96.
[34] Tu, J., Song, W. and Carlson, M. (1996) 16, 4199–4206.