Glycogen-binding domains in protein phosphatases

Printen *et al.* (1) have identified a novel glycogen-sensitive subunit of protein phosphatase 1 (PP1G) which they called PTG because it performs protein targeting to glycogen. PTG is capable of binding the primary enzymes involved in hormonal regulation of glycogen metabolism in insulin-sensitive tissues (1). We would like to comment that, using sequence analysis, it is possible to (i) predict the location and three-dimensional structure of the putative high affinity glycogen-binding site on PTG and other PP1Gs (Fig.1) as well as to (ii) confine the binding site for the catalytic subunit PP1C on these proteins.

The entire yeast genome encodes four PP1G homologues that have been implicated in glycogen synthesis (2). They have only two conserved regions in common with the animal PP1Gs. Subjecting one of these regions, located in the central part of PTG, to iterative motif and profile searches (3), in addition to human PTG, a distinct human homologue, EST R10183, can be retrieved. Furthermore, numerous high affinity starch-binding domains of distinct glycohydrolases (Fig.1) were identified. For several of them, the three-dimensional structure has been determined. The N- and C-terminal location of the domain in some of the glycohydrolases as well as the three-dimensional structures allow the domain borders to be defined and the fold to be predicted as a β-sandwich. Mapping the conserved residues onto the predicted structure reveals the exposed polar amino acids that are the candidates for the direct interaction with glycogen (Fig 1).

The second region of significant similarity shared by all PP1Gs starts at position 60 in PTG (motif in one letter code: [R/K][R/S/N]VxF[A/D]; x is any amino acid; [K/R] means R or K) with a probability of chance matching $p=1.7\times10^{-14}$ as measured using the MACAW program (4)). The PP1C-binding site on the rabbit PP1 Gm subunit has been located in a region that includes this motif (5). Thus, our sequence analysis suggests, that at the molecular level, common mechanisms exist for all PP1Gs to target enzymatic activities to glycogen and to bind PP1C. In contrast, other features such as phosphorylation sites as well as tissue and compartment-specific activities of distinct PP1Gs (1) are not shared by all family members and should mostly be realized in the remaining parts of the molecules that are dissimilar to each other.

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Fig. 1 Alignment of PTG and some related PP1G subunits with selected starch-binding domains of glycohydrolases. Protein and species abbreviations (first column) are taken from or adapted to the SWISS-PROT database. For unique identification, database accession numbers and positions in the respective sequences are given (second and third columns). Numbers in between the blocks denote the intervening residues. The starch-binding domains (6,7) of glycoamylase G2 from *Aspergillus nidulans* (PDB code 1KUL) and cyclodextringlycosyltransferase from *Bacillus circulans* (PDB code 1CGT) are taken from the Protein Databank of known three-dimensional structures. Secondary structure prediction of the animal PP1Gs (above alignment) is in agreement with the β-strands a-g obtained from 1KUL (beneath alignment). Hydrophobic positions are shown in bold. Several of the conserved residues (highlighted) cluster together in the structure with the polar side chains being candidates for direct interaction with glycogen.

The similarity of PP1Gs and the starch-binding domain of Amyg_Rhior can be obtained using standard methods such as Blast (2, 8) with a probability of a chance match $p=4.6.10^{-8}$. A similarity of the N-terminal domain in Amyg_Rhior and other starch-binding domains of glycohydrolases has already been noted (9). Motif and profile searches (for strategies see ref. 3) quantify these findings and identify a large superfamily of β-sandwich domains resembling structurally related fibronectin type 3 repeats that also occur as carbohydrate-binding domains in various glycohydrolases (see 10 and refs. therein). For more data including the search protocol, a full-length alignment with more family members and three-dimensional models of the domains see http://www.bork.embl-heidelberg.de/pp1g/pp1g.html.