Biochimie 118 (2015) 207–220

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Review

Structure-function relationships in mammalian histidine-proline-rich glycoprotein

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ARTICLE INFO

Article history:
Received 22 June 2015
Accepted 22 September 2015
Available online 26 September 2015

Keywords:
AMP deaminase
Anthropoidea evolution
Histidine-proline-rich glycoprotein
Histidine-rich glycoprotein
Metallochaperone
Zinc-binding motif

ABSTRACT

Histidine-proline-rich glycoprotein (HPRG), or histidine-rich glycoprotein (HRG), is a serum protein that is synthesized in the liver and is actively internalised by different cells, including skeletal muscle. The multidomain arrangement of HPRG comprises two modules at the N-terminus that are homologous to cystatin but void of cysteine proteinase inhibitor function, and a second half consisting of a histidine-proline-rich region (HPRR) located between two proline-rich regions (PRR1 and PRR2), and a C-terminus domain.

HPRG has been reported to bind various ligands and to modulate angiogenesis via the histidine residues of the HPRR. However, the secondary structure prediction of the HPRR reveals that more than 98% is disordered and the structural basis of the hypothesized functions remains unclear. Comparison of the PRR1 of several mammalian species indicates the presence of a conserved binding site that might coordinate the Zn^{2+} ion with an amino acid arrangement compatible with the cysteine-containing site that has been identified experimentally for rabbit HPRG. This observation provides a structural basis to the function of HPRG as an intracellular zinc chaperone which has been suggested by the involvement of the protein in the maintenance of the quaternary structure of skeletal muscle AMP deaminase (AMPD). During Anthropoidea evolution, a change of the primary structure of the PRR1 Zn^{2+} binding site took place, giving rise to the sequence M-S-C-S/L-S/R-C that resembles the MxCxxC motif characteristic of metal transporters and metallochaperones.

Contents

Abbreviations: AHSG, α_2-Heremans-Schmid glycoprotein; AMPD, AMP deaminase; CCS, copper chaperone for SOD1; CD, circular dichroism; CRP, cystatin-related protein; EXAFS, extended x-ray absorption fine structure; GAGs, glycosaminoglycans; HBA, human albumin; HPRG, histidine-proline-rich glycoprotein; HPRR, histidine-proline-rich region; HRG, histidine-rich glycoprotein; NCBI, National Center for Biotechnology Information; PPII, polyproline-II helix; PRR1, proline-rich region 1; PRR2, proline-rich region 2; SOD1, Cu, Zn superoxide dismutase; TGF-β, tumor growth factor β.

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http://dx.doi.org/10.1016/j.biochi.2015.09.024
0300-9084/

1. Introduction

Histidine-proline-rich glycoprotein (HPRG) is a glycoprotein of 70–75 kDa, present at a relatively high concentration in the plasma of vertebrates (100–150 μg/mL in humans), that is also found at lower concentrations in infant urine, colostrum and milk. It is produced in the liver, but it has also been reported to be synthesized by monocytes, macrophages and megakaryocytes [1]. However, no HPRG mRNA was found in immune tissues by RT-PCR analysis, indicating that the HPRG present in immune cells is acquired from plasma [2]. A protein very similar in sequence to HPRG co-purified with rabbit skeletal muscle AMP deaminase (AMPD) preparation [3]. It has been later demonstrated that the HPRG present in rabbit skeletal muscle is acquired from plasma since skeletal muscle cells do not synthesize the protein [4].

HPRG appears to be involved in several processes, such as blood coagulation and fibrinolysis, immune complex clearance, cell adhesion, cell migration and transport of metal ions, due to its ability to bind various ligands such as phospholipids, fibrinogen, plasminogen, heparin, heparan sulfate, tropomyosin, heme and the divalent metal ions zinc, copper, mercury, cadmium and nickel [5]. However, to date the physiological role of HPRG in plasma has not clearly been determined. HPRG has also been shown to have a role in the angiogenesis process since it inhibits the antiangiogenic properties of thrombospondin-1 [6]. In contrast, the inhibition of angiogenesis [7] was achieved upon the zinc-dependent binding of HPRG to heparan sulfate on endothelial cells.

Recent data obtained from HPRG deficient (HPRG−/−) mice [reviewed in 8] reported a shorter plasma prothrombin time, strongly suggesting that HPRG may inhibit coagulation also *in vivo* [9]. In HPRG−/− mice fibrin clots are also proteolyzed more rapidly than in wild-type mice, indicating that HPRG may act as an antifibrinolytic protein *in vivo* [9]. Moreover, HPRG−/− mice are more easily infected by bacteria and fungi [10,11], in accord with the data indicating that HPRG can mediate the lysis of microbes *in vitro*. Recent studies have also reported that HPRG might also have a role in tumor growth [12]. Consistent with the observations that, when overexpressed, HPRG reduced the necrotic areas in allografted tumors, HPRG−/− mice grow larger tumors with increased necrotic areas, compared to wild-type mice [13,14].

The interaction of HPRG with metal ions and various proteins, sulfated polysaccharides and phospholipids is based on *in vitro* studies. The observation that HPRG purified by different methods can exhibit different properties raises, however, questions over the validity of the numerous *in vitro* biological activities ascribed to plasma HPRG. In comparison with the protein purified following the most commonly used procedure involving chromatography on phosphocellulose, human plasma HPRG purified using cobalt/anion exchange chromatography retained the ability to bind to heparin and phosphatidate but not with necrotic cells or other membrane phospholipids [15]. These evidences suggest that some of the functions ascribed to HPRG might indeed derive from the co-purification of its true ligands or other unrelated molecules.

2. Structural analysis of HPRG

2.1. The HPRG multidomain structure

The sequence of human HPRG derived from its cDNA [16] was the first sequence deposited, followed by the rabbit HPRG sequence, deposited in the GenBank with the accession number AAC48516.1 [17].

Borza *et al.* [17] showed that the structure of rabbit plasma HPRG is similar to that of the human protein [16]: the molecule is composed by two modules homologous to cystatin at the N-terminus, a histidine-proline rich region located between two proline-rich regions and a C-terminal domain. More recently, the transcribed RNA sequence and the predicted amino acid sequence of rabbit HPRG have been deposited in GenBank under the accession number XP_008264798.1. Fig. 1 shows the alignment of the two rabbit HPRG sequences. A 95% amino acid identity and a 2% gap (i.e. the space introduced into an alignment to compensate for insertions and deletions) between the two sequences were calculated by the National Center for Biotechnology Information (NCBI) BLAST software (blast.ncbi.nlm.nih.gov). Differences in 28 amino acids can be located in the C-terminal half of the protein. The observation that the amino acid sequence of rabbit skeletal muscle HPRG fragments obtained by trypsinization [18] well corresponds to the genome predicted sequence of rabbit HPRG indicates that the XP_008264798.1 sequence is the correct one. Since previous papers that described the multi-domain structure of HPRG were based on what is probably an incorrect amino acid sequence for the rabbit protein (AAC48516.1) [8,19,20], it is necessary to reconsider the domain structure using the newly published sequence data for the protein, starting with a comparison of the human HPRG sequence (AAI50592.1) and the genome-predicted sequence of rabbit HPRG (XP_008264798.1) (Fig. 2A). Our new alignment shows that the two orthologs share 292 identical amino acids (60% identity), 385 semi-conserved and conserved amino acids (68% positives) and 53 gapped amino acids (9% gap) over 545 residues, as calculated by the NCBI BLAST software. The multidomain arrangement of the two proteins that is depicted in Fig. 2B comprises two modules that are homologous to cystatin (N1 and N2), and a second half consisting of a first proline-rich region (PRR1), a histidine-proline rich region (HPRR), a second proline-rich region (PRR2) and a C-terminus domain (C-term). The amino acid residues of the different domains of the human and rabbit HPRG molecules have been used to calculate the percentages of amino acid identities and conservative substitutions (positives) within the different domains that are reported in Table 1. The data show that a high homology occurs at the two amino terminal cystatin-like domains N1 and N2, with 84% positives for both the N domains and at the carboxyl terminal region with 77% positives. In these three domains, the alignment of rabbit and human HPRGs do not show any gap. A lower homology is observed over the PRR1 (54%) and PRR2 (71%) with a 5% gap in the latest. The HPRR presents the biggest difference between the two proteins as revealed by the 38% gap and the low number of the conserved amino acids (39% positives).