Vol. 200, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1994 Pages 78-82

BOVINE HISTIDINE-RICH GLYCOPROTEIN IS A SUBSTRATE FOR BOVINE PLASMA FACTOR XIIIa

Torben Halkier*, Hanne Andersen, Astrid Vestergaard and Staffan Magnusson◇

Department of Molecular Biology and Plant Physiology
University of Aarhus, DK-8000 Aarhus C, Denmark

Received February 24, 1994

Histidine-rich glycoprotein was purified from bovine plasma and the identity of the protein confirmed through amino acid sequencing. Activated bovine factor XIIIa catalyzed the incorporation of 1 nmol of 1,4-[^{14}C]putrescine into 1 nmol of bovine histidine-rich glycoprotein,showing that histidine-rich glycoprotein has the ability to participate in transglutaminase-catalyzed reactions *in vivo*.

Histidine-rich glycoprotein is a single-chained plasma protein of unknown physiological function with an M_r of 81 kDa (1). However, a variety of properties and functions *in vitro* have been described since the first purification of histidine-rich glycoprotein in 1972 (2,3). The ability of histidine-rich glycoprotein to bind to heparin (3), to the lysine-binding site of kringle 1 in plasminogen (4) and to divalent cations (5) has been utilized in purification procedures (4,6-8). In addition, histidine-rich glycoprotein mediates inhibition of autorosetting of autologous erythrocytes by T-lymphocytes (1, 9-11), it inhibits contact activation of blood coagulation (7), it promotes t-PA-catalyzed plasminogen activation (12) and it binds fibrinogen and fibrin (13). These observations *in vitro* indicate functions of histidine-rich glycoprotein *in vivo* in blood coagulation, fibrinolysis and immunoregulation (4,7,11-13). Thus, it is interesting to note that the polypeptide chain in human histidine-rich glycoprotein, consisting of 507 amino-acid residues, contains regions that share homology with other plasma proteins, *e.g.* antithrombin III and high-molecular-mass kininogen (14). Recently the arrangement of the disulphide bridges in bovine histidine-rich glycoprotein was determined (15).

* To whom correspondence and reprint requests should be addressed at Novo Nordisk A/S, Novo Allé, DK-2880 Bagsvaerd, Denmark.

◇ Deceased.

0006-291X/94 $5.00

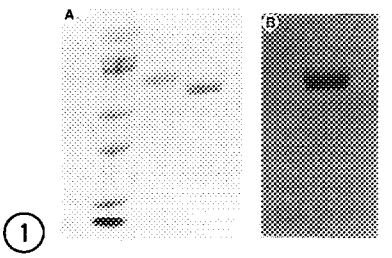
In addition to its binding to fibrinogen and fibrin, histidine-rich glycoprotein becomes incorporated into fibrin clots in a Ca^{2+}-ion dependent manner (13). This could be due to covalent cross-linking of histidine-rich glycoprotein to fibrin catalyzed by factor XIIIa. The present study was undertaken to investigate the possibility that histidine-rich glycoprotein is a substrate for factor XIIIa.

Materials and Methods

All standard chemicals were of analytical grade and obtained from Aldrich (Steinheim, Germany), Sigma (St. Louis, MO, USA), Merck (Darmstadt, Germany) or Riedel-deHaën (Seelze, Hannover, Germany). 1,4-[^{14}C]putrescine, iodo 2-[^{14}C]acetic acid and Amplify were from Amersham International (Amersham, UK). Trypsin (TPCK-treated) was from Worthington (Freehold, NJ, USA). Sephadex G-50 superfine was from Pharmacia-LKB (Uppsala, Sweden). Column materials for reversed phase HPLC were Spherisorb 5s ODS2 (Phase Separations, Deeside, UK), Vydac C_{18} 5 μm (Hesperia, CA, USA) and Nucleosil C_{18} 5 μm (Macherey-Nagel, Düren, Germany). Reagents and solvents for amino acid sequencing were from Rathburn (Walkerburn, Scotland) except Quadrol which was from Pierce (Rockford, IL, USA). Human thrombin was a kind gift from Dr. John W. Fenton II (State Department of Health, Albany, NY, USA). Normal human plasma was from one of us (TH) and prepared from 250 ml blood drawn into 4 ml 0.25 M disodium-EDTA-dihydrate, pH 7.5. Fresh bovine blood was collected at the local slaugtherhouse according to Halkier and Magnusson (16). Bovine and human histidine-rich glycoprotein were purified according to Vestergaard *et al.* (7) while bovine plasma factor XIII was purified according to Halkier and Magnusson (16). Reduced and S-carboxymethylated (17) bovine histidine-rich glycoprotein (50 mg) was digested with trypsin (16h, 37 °C, enzyme:substrate 1:100 (w/w)) and the resulting peptides separated using a Sephadex G-50 superfine column (2.5 cm x 100 cm) equilibrated in 50 mM NH_4HCO_3. The resulting pools were lyophilized prior to further purification by reversed phase HPLC using a Hewlett Packard 1084B liquid chromatograph. All peptides sequenced were initially fractionated on Nucleosil C_{18} resin employing gradients of freshly distilled 96% ethanol in 0.1% aqueous TFA followed by repurification on Vydac C_{18} resin using gradients of 80% acetonitrile containing 0.08% TFA in 0.1% aqueous TFA. Purified peptides were sequenced using a Beckman 890C sequencer and the pth-amino acids were identified by reversed phase HPLC. Factor XIIIa-catalyzed incorporation of 1,4-[^{14}C]putrescine into histidine-rich glycoprotein was studied using the filter paper assay described by Curtis and Lorand (18) except that casein was substituted with histidine-rich glycoprotein (enzyme:substrate 1:75 (mol:mol)). SDS-PAGE was carried out according to Laemmli (19). Fluorography was carried out for 3 days at -80 °C using vacuum dried gels that had been soaked in Amplify for 30 min before drying.

Results and Discussion

Homogeneity of purified bovine as well as human histidine-rich glycoprotein were confirmed through N-terminal amino acid sequencing and SDS-PAGE. Bovine histidine-rich glycoprotein migrates as a closely spaced doublet in reduced SDS-PAGE with a M, of 80 kDa (7,8) (Figure 1). Human histidine-rich glycoprotein migrates

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    AVNPTGCDAVE PVAVRALDLI NK            VADAHL DKVESIAVYY
  1 VSPTDCSAVE PEAEKALDLI NKRRRDGYLF QLLRIADAHL DRVENTTVYY  50
    * ** * *** * *  ***** **             ***** * **   ***

    LVLDVKESDC PVLSRKHWDD CELNVSRYPS ETVIGQCKVI AITLLAGPED
 51 LVLDVQESDC SVLSRKYWND CEPPDSRRPS EIVIGQCKVI ATRHSHESQD 100
    ***** ****  ***** * * **   ** ** * ******** *       *

    LR       T VGEYTEGANT RIIDSPVLFD FFEDTTLY
101 LRVIDFNCTT SSVSSALANT K--DSPVLID FFEDTERYRK QANKALEKYK 148
    **       *       ***    ***** * *****  *

     ENSDFAPFR             GGEGTSYFL DFSVRNCSSH HFPRHSHIFG
149 EENDDFASFR VDRIERVARV RGGEGTGYFV DFSVRNCPRH HFPRHPNVFG 198
     *  *** **             ***** **  *******  * *****   **
                                      ◊
    FCRADLFYDV EASDLETPKD IVTNCEVFNL K   NFSAVQ HHLGRPFHWG
199 FCRADLFYDV EALDLESPKN LVINCEVFDP QEHENINGVP PHLGHPFHWG 248
    ********** ** *** **   * *****       *   *   *** *****

    GH                                              APLPFPP
249 GHERSSTTKP PFKPHGSRDH HHPHKPHEHG PPPPPDERDH SHGPPLPQGP 298
    **                                              ***  *
                                ◊
    PGLR               G NHRPPHDNSS DEHHN
299 PPLLPMSCSS CQHATFGTNG AQRHSHNNNS SDLHPHKHHS HEQHPHGHHP 348
    * *                *   *  * * *    *

349 HAHHPHEHDT HRQHPHGHHP HGHHPHGHHP HGHHPHGHHP HCHDFQDYGP 398

399 CDPPPHNQGH CCHGHGPPPG HLRRRGPGKG PRPFHCRQIG SVYRLPPLRK 448

449 GEVLPLPEAN FPSFPLPHHK HPLKPDNQPF PQSVSESCPG KFKSGFPQVS 498

499 MFFTHTFPK                                           507
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Figure 1.
Panel A: SDS-PAGE (10-20% gradient gel) of purified bovine histidine-rich glycoprotein in the presence and absence of dithioerythritol. Lane 1: molecular weight standards (from top to bottom 180 kDa, 120 kDa, 92 kDa, 60 kDa, 43 kDa, 26 kDa and 14 kDa); lane 2: bovine histidine-rich glycoprotein in the presence of dithioerythritol; lane 3: bovine histidine-rich glycoprotein in the absence of dithioerythritol. *Panel B:* Fluorography of 1,4-[^{14}C]putrescine labeled bovine histidine-rich glycoprotein. SDS-PAGE was performed as above in the absence of dithioerythritol. Fluorography was carried out as descibed in Materials and Methods.

Figure 2.
Alignment of the peptide sequences obtained from bovine histidine-rich glycoprotein with the amino acid sequence of human histidine-rich glycoprotein (14). In the position corresponding to position 46 both Ile and Thr were detected. In the position corresponding to position 111 both Val and Asn were detected. ◊ indicates the positions of a possible N-glycosylation sites. Identical residues in the two sequences are indicated with a * below the human sequence and - indicates gaps introduced to obtain an optimal alignment.

slightly faster (M_r of 75 kDa) as a single band (results not shown). N-terminal amino acid sequencing gave results identical to the published N-terminal sequences (7,8,14). Through peptide sequencing it was clearly established that bovine histidine-rich glycoprotein shares homology with human histidine-rich glycoprotein (Figure 2).

Factor XIIIa-catalyzed incorporation of 1,4-[^{14}C]putrescine into bovine histidine-rich glycoprotein was investigated and the time dependence of the incorporation is shown in Figure 3. The incorporation depended on the presence of Ca^{2+}-ions as well as on the presence of factor XIIIa. The incorporation was found to be higher at 37 °C than at room temperature. It was found that the incorporation was independent of the presence of Zn^{2+}-ions which histidine-rich glycoprotein is known to bind. The

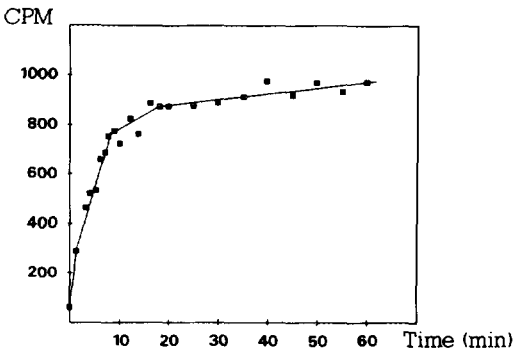

Figure 3.
Incorporation of 1,4-[^{14}C]putrescine into bovine histidine-rich glycoprotein. To 160 μl bovine plasma factor XIII (0.1 mg/ml in 10 mM Tris-HCl, 150 mM NaCl, pH 7.5) were added 40 μl thrombin (150 U/ml) and 80 μl 40 mM $CaCl_2$, 10 mM Tris-HCl, 150 mM NaCl, pH 7.5. Following mixing activation took place for 30 min at 37 °C before addition of 40 μl 1,4-[^{14}C]putrescine (25 μCi/ml) and 160 μl bovine histidine-rich glycoprotein (3.6 mg/ml in 10 mM Tris-HCl, 150 mM NaCl, pH 7.5). Following mixing incubation was carried out at 37 °C. At the indicated time points 15 μl of the mixture was spotted onto Whatman 3MM filters that were treated as desribed (18).Incorporation of 1,4-[^{14}C]putrescine was measured by liquid scintillation with a counting efficiency of 95%. Equivalent incorporation studies carried out in the absence of any of the components resulted in negligible amounts of radioactivity incorporated.

concentration of Zn^{2+}-ions was varied between 0 and 50 μM using increments of 5 μM. Calculations show that the amount of radioactivity incorporated equals 1.0 mol 1,4-[^{14}C]putrescine per mol bovine histidine-rich glycoprotein. Thus, it seems likely that bovine histidine-rich glycoprotein contains one glutamine residue that is susceptible to factor XIIIa-catalyzed incorporation of 1,4-[^{14}C]putrescine. Human histidine-rich glycoprotein was also labeled following incubation with bovine factor XIIIa in the presence of 1,4-[^{14}C]putrescine (results not shown). Figure 1 includes fluorography of 1,4-[^{14}C]putrescine labeled bovine histidine-rich glycoprotein after SDS-PAGE.

The presence of a glutamine residue susceptible to factor XIIIa-catalyzed incorporation of 1,4-[^{14}C]putrescine is a prerequisite for covalent incorporation of histidine-rich glycoprotein into fibrin clots. Combining this observation with the previous observation of a Ca^{2+}-ion dependent incorporation of histidine-rich glycoprotein into fibrin clots (13) and the observation that immobilized histidine-rich glycoprotein increases the efficiency of t-PA-catalyzed plasminogen activation (12) points to the possibility of histidine-rich glycoprotein as a fibrin-linked plaminogen activator promotor.

Acknowledgments

This work has supported financially by the Danish Cancer Society and the NOVO Foundation through grants to TH and by the Carlsberg Foundation through a research scholarship awarded to HA.

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