THE N-TERMINAL SEQUENCE OF HUMAN PLASMA HISTIDINE-RICH GLYCOPROTEIN HOMOLOGOUS TO ANTITHROMBIN WITH HIGH AFFINITY FOR HEPARIN

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1. Introduction

Histidine-rich glycoprotein (HRG) is one of the plasma glycoproteins whose function is not yet known. This protein was first isolated in [1]; some antifibrinolytic effect of this protein has been suggested [2]. Antithrombin is one of the major plasma proteinase inhibitors and is known to have high affinity for heparin [3]. Lysyl residue(s) of antithrombin is reported to be involved in the interaction with heparin [3]. However, its location in the amino acid sequence is not known. The amino acid sequence of antithrombin [4] has been shown to be highly homologous to α_1-proteinase inhibitor and ovalbumin, except the N-terminal region [5,6]. Here, we will describe a newly isolated plasma protein which has strong heparin-binding ability comparable to antithrombin; is possibly identical with HRG and show that this protein has a homologous N-terminal sequence to that of N-terminal region of antithrombin. The results suggest that the putative heparin-binding site of the 2 proteins is located at the N-terminal homologous position.

2. Materials and methods

2.1. *Isolation of HRG*

A new heparin-binding protein (HBP) was purified from human plasma by heparin–agarose affinity chromatography, followed by DEAE–Sephadex chromatography (T. K., S. O., T. O., unpublished). The heparin–agarose procedure was as in [7]. Fractions containing HBP were pooled, dialyzed with 50 mM Tris–10 mM Na-citrate–20 mM NaCl (pH 8.0) and applied to a column (2.5 × 20 cm) of DEAE–Sephadex equilibrated with the same buffer. After washing the column with the equilibration buffer, HBP was eluted with a linear gradient of NaCl (20–520 mM) in the same buffer (total gradient vol., 600 ml).

The purified protein gave a single band by SDS–polyacrylamide gel electrophoresis. Amino acid analysis of this protein showed the unusually high content of proline and histidine accounting for >20% of the total number of residues, and the amino acid composition was very close to those reported in [1] as HRG. High affinity of HRG for heparin was also described in [1]. Identity of this protein as HRG was further confirmed by double immunodiffusion method using anti-human HRG antiserum prepared as in [1].

2.2. *Protein sequence determination*

HRG was reduced and carboxymethylated according to [8]. The N-terminal amino acid sequence analysis of Cm-derivative (7.7 mg) was performed by automated Edman degradation [9] in a JEOL JS-47 K sequence analyzer using 0.25 M Quadrol buffer and double-coupling program at the first step. Phenylthiohydantoin derivatives were semi-quantified by measuring the absorbance at 269 nm, identified by thin-layer chromatography on Kiesel gel F-254 sheets (Merck) [10,11] and confirmed by amino acid analysis after back hydrolysis to free amino acids at 150°C for 6 h in 6 N HCl containing 0.2% $SnCl_2$ [12].

3. Results and discussion

Twenty-two steps of the Edman degradation of the *S*-carboxymethyl cysteinyl HRG gave the sequence as:

Val–Ser–Pro–Thr–Asp–Ser–(Ser)–Ala–Val–Glu–Pro–Glu–Ala–Glu–Lys–Ala–Leu–Asx–Leu–Ile–Asx–Lys.

The repetitive yield, calculated from the yields of the

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HRG	^{1}Val	Ser	Pro	Thr	Asp	Ser(Ser)	Ala	Val	Glu	Pro	Glu	Ala	-	-	Glu	Lys	Ala	Leu	Asx	Leu	Ile	Asx	Lys^{22}	
Antithrombin	^{24}Arg	Ser	Pro	-	Glu	Lys	Lys	Ala	Thr	Glu	Asp	Glu	Gly	Ser	Gln	Glu	Lys	Ile	Pro	Glu	Ala	Thr	Asn	Arg^{46}

Fig.1. Comparison of the N-terminal sequence of histidine-rich glycoprotein (HRG) with the sequence of antithrombin [4]. Numbering is based on the sequence of each protein. Amino acids enclosed in boxes represent identical residues and functionally conserved residues, designated [13] from chemical similarity and accepted point-mutation data. Dash indicates the introduction of a gap in the sequence. Serine residue in parentheses is tentative identification.

alanine residues in positions 8, 13 and 16 was found to be 98%. The N-terminal sequence, Val–Ser–Pro, is identical to that in [2].

Comparison of the N-terminal sequence of HRG with the sequence of antithrombin starting with the arginine residue-24 (this arginine is also the starting residue when compared with α_1-proteinase inhibitor [6]) reveals that there is a significant degree of sequence homology between these 2 plasma proteins which are common in the property of high affinity for heparin (fig.1).

HRG, however, showed neither thrombin inhibitory activity nor heparin cofactor activity (T. K., unpublished). These results show that the 2 apparently functionally unrelated plasma proteins with heparin-binding ability in common have a homologous sequence at the N-terminal regions of both proteins.

Extensive sequence homology between antithrombin and α_1-proteinase inhibitor has been shown all through the peptide chains of both proteins except the N-terminal regions [6]. According to computer analysis of the sequence homology of ovalbumin with antithrombin and α_1-proteinase inhibitor [5], the N-terminal portions of the 2 homologous proteinase inhibitors are not related, and there seem to be no proteins, in the Atlas data base [13], which are homologous to the N-terminal 48 residues of antithrombin. These facts show that the N-terminal sequence of HRG reported here is homologous to the very unique portion of antithrombin where even α_1-proteinase inhibitor reveals no homology. The significance of this sequence homology is discussed below.

The binding of heparin to antithrombin molecule extremely enhances the protease inhibitory activity of the inhibitor [3] and this heparin cofactor activity is physiologically very important, for a patient with hereditary abnormal antithrombin of the normal progressive antithrombin activity and low heparin cofactor activity suffers from recurrent thrombophlebitis [14]. Although lysyl residue(s) of antithrombin is reported to be involved in the interaction with heparin [3], its location in the amino acid sequence is not known. Thus, the sequence homology at the N-terminal region of HRG and antithrombin is of interest from a heparin-binding point of view. Furthermore, it is often observed in plasma proteins that the binding site is located at the N-terminal portion, and the biologically active site at the C-terminal portion: Ca-binding site and active serine site of vitamin K-dependent coagulation factors [15], and lysine-binding site and active serine site of plasmin [16]. The reactive sites of antithrombin and α_1-proteinase inhibitor are also located at the C-terminal homologous position [6]. From these observations it is likely that the heparin-binding sites of antithrombin and HRG are also located at the N-terminal portion where the sequence of the 2 proteins is homologous.

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