The heparin-binding site(s) of histidine-rich glycoprotein as suggested by sequence homology with antithrombin III

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A high degree of sequence homology has been found between the N-terminal region of histidine-rich glycoprotein (HRG) and that of antithrombin III (AT III) where the putative heparin-binding site of AT III is located. The amino acid residue at the position corresponding to Arg-47 of AT III that is essential for the heparin-binding was also arginine (Arg 23 and 78) in the homologous sequences of HRG. These observations strongly suggest that the heparin-binding sites of HRG and AT III are evolutionarily related. There was no apparent sequence similarity between the remaining about 70% portions of the two proteins.

Histidine-rich glycoprotein *Antithrombin III* *Heparin* *Heparin-binding site* *Sequence homology*

1. INTRODUCTION

Antithrombin III (AT III) and histidine-rich glycoprotein (HRG) are plasma glycoproteins that have a high affinity for heparin. The importance of heparin-accelerated anticoagulant activity (heparin-cofactor activity) of AT III has been well recognized by the observation that a congenital defect of AT III having normal antithrombin activity but no heparin-binding ability results in recurrent thrombophlebitis [1]. It has also been suggested that the heparin-binding property of HRG may be of physiological importance as a modulator of AT III and heparin cofactor II [2–4].

In [5], we reported that the N-terminal 22 amino acid sequence of HRG is homologous to that of AT III, and suggested that the putative heparin-binding sites of the 2 proteins were located in the N-terminal region of each protein. We also showed that Arg 47 of AT III is essential for heparin binding [6], supporting the original suggestion [5].

Recently, the complete amino acid sequence of human HRG has been determined by cDNA cloning [7]. Here, the expanded N-terminal sequence of HRG (residues 1–146) is shown to be highly homologous with the N-terminal region of AT III. This region includes the heparin-binding site of AT III and strongly suggests that arginine present in the same position in HRG as in AT III is also involved in heparin binding.

2. RESULTS AND DISCUSSION

Fig.1 shows a comparison of the amino acid sequences of HRG (residues 1–64 and 65–146) and AT III (residues 24–139). The residues that are identical or conserved between HRG and AT III are shown in blocks. In comparing the N-terminal region (residues 1–64) of HRG with AT III, 27 residues out of 67 were either identical or highly conserved (40% homology). A comparison of residues 65–146 of HRG with AT III showed that 43 residues out of 117 were identical or conserved (37% homology). There was no apparent homology in the remaining portion (70%) of the 2

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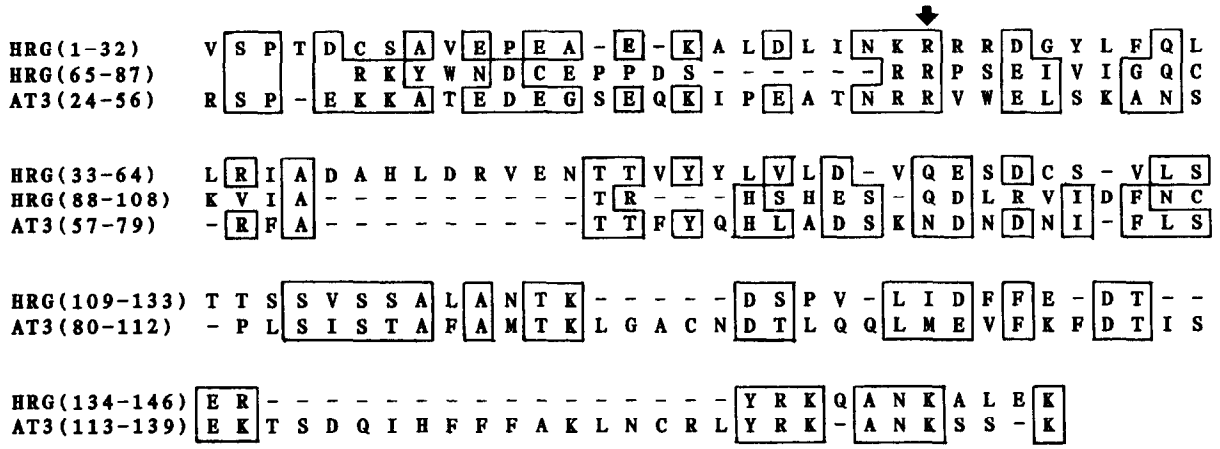

Fig.1. Comparison of the amino acid sequences of HRG and AT III. HRG, histidine-rich glycoprotein [5,7], AT 3, antithrombin III [13–16]. The numbers in parentheses are residue number in sequence of each protein. Identical or conserved residues between HRG and AT 3 are enclosed in boxes. Gaps are included for the best alignment. An arrow shows Arg 47 that has been replaced by cysteine in 'Antithrombin III Toyama' [6]. Single-letter code for amino acids: Ala, A; Arg, R; Asn, N; Asp, D; Cys, C; Gln, Q; Glu, E; Gly, G; His, H; Ile, I; Leu, L; Lys, K; Met, M; Phe, F; Pro, P; Ser, S; Thr, T; Trp, W; Tyr, Y; Val, V.

proteins. These results extend our previous observation that these 2 proteins are highly homologous in their N-terminal regions [5].

Recently, the structural abnormality of antithrombin III Toyama was shown to be due to a lack of heparin-binding ability and this was due to a replacement of Arg 47 by cysteine [6]. These data provided good evidence to support the concept that Arg 47 was an essential residue for heparin binding to AT III. A striking feature of the present sequence homology between HRG and AT III is that Arg 47 is present in a homologous position in both proteins. Furthermore, there are 2 arginine residues (Arg 23 and Arg 78) in HRG that are aligned at this position. These observations suggest that there could be 2 heparin-binding sites in HRG. However, there is a large deletion just prior to Arg 78 (between Ser 76 and Arg 77) in this alignment.

The interaction between each of these proteins and heparin probably involves one or more of the negatively charged sulfate groups in the penta- to octasaccharide units in the heparin molecule that are important for its binding to AT III [8–10]. Therefore, it is also likely that in addition to Arg 47, several additional positively charged groups in AT III may participate in its interaction with heparin. From the sequence homology between HRG and AT III, it may be possible that Lys 39, Arg 46 and Arg 57 of AT III, and by analogy, Lys 15, Lys 22, Arg 23, Arg 34, Arg 76, Arg 77 and Lys 87 of HRG are also involved in the binding of heparin.

In spite of the high sequence homology of these 2 proteins at their N-terminal regions, there was no apparent sequence similarity between the remaining portions of the 2 proteins. In the C-terminal portion of AT III there is a high homology with α_1-antitrypsin, α_1-antichymotrypsin and ovalbumin [11,12]. These observations strongly suggest that HRG and AT III are evolutionarily related only in their heparin-binding region or domain.

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