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Cystatin domains in alpha-2-HS-glycoprotein and fetuin

Andrzej Elzanowski, Winona C. Barker, Lois T. Hunt and Elizabeth Seibel-Ross

Protein Identification Resource, National Biomedical Research Foundation, Georgetown University Medical Center, 3900 Reservoir Road, N.W., Washington, DC 20007, USA

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We have found that chain A of alpha-2-HS-glycoprotein contains two cystatin domains that show closest similarity to those of kininogen. Most likely, the two proteins diverged after the primary duplication of a single cystatin domain as the two cystatin domains of alpha-2-HS-glycoprotein are more similar, especially in disulfide bonding, to the corresponding domains of kininogen than to each other. We also propose that the carboxyl-terminal (non-cystatin) parts of kininogen and alpha-2-HS-glycoprotein contain homologous segments. We suggest that alpha-2-HS-glycoprotein may act as an inhibitor of the cysteine proteinases responsible for bone resorption. We have also found that fetuin is closely related to alpha-2-HS-glycoprotein.

Cysteine proteinase inhibitor; α_2-HS-glycoprotein; Kininogen; Plasma protein; Bone resorption; Fetuin

1. INTRODUCTION

The cystatin superfamily includes inhibitors of cysteine proteinases found in various tissues and body fluids of mammals and birds [1–3]. These inhibitors are likely to be found also in other vertebrates, as they are probably present in a chondrichthyan fish [4]. Cystatins, which are extracellular, have about 115 amino acids and two disulfide bonds. Homologous intracellular inhibitors with 100 amino acids and no disulfide bonds are known as stefins. Ohkubo et al. [5] established that the carboxyl two-thirds of the heavy chain of kininogen contains two domains similar and clearly homologous to cystatins and stefins. Salvesen et al. [6] demonstrated that the remaining amino-terminal domain of kininogens is also homologous, although less similar, to cystatins. Each of the three domains is encoded by three contiguous exons and both the gene structure and the protein sequence suggest that the kininogen gene originated by two successive duplications [7,8]. Duplicated more recently was the carboxyl-terminal cystatin domain of the two-domain intermediate form, because the second and third domains of the present kininogen are more similar to each other than either is to the amino-terminal domain. The first domain does not show any inhibitory activity whereas the other two domains inhibit papain and cathepsin L, but only the second (middle) domain inhibits calpain [6].

We discovered that at least two cystatin domains are present in chain A of alpha-2-HS-glycoprotein (alpha-2-HS). The alpha-2-HS molecule is composed of two chains; the sequence of the longer chain A was determined by Yoshioka [9] and that of chain B by Gejyo et al. [10]. Recently, Lee et al. [11] determined the sequence of the single mRNA transcript coding for the precursor, including the signal sequence, both chains, and the connecting peptide. Alpha-2-HS occurs in plasma and, at levels much higher than other plasma proteins, in the matrix of bone and dentine; this association with mineralized tissues and the affinity for calcium and barium ions suggest some role in mineral balance. Alpha-2-HS is known also as an acute phase negative reactant. The function of alpha-2-HS remains unknown.

We also found that the known sequence frag-

Correspondence address: A. Elzanowski, Protein Identification Resource, National Biomedical Research Foundation, Georgetown University Medical Center, 3900 Reservoir Road, N.W., Washington, DC 20007, USA

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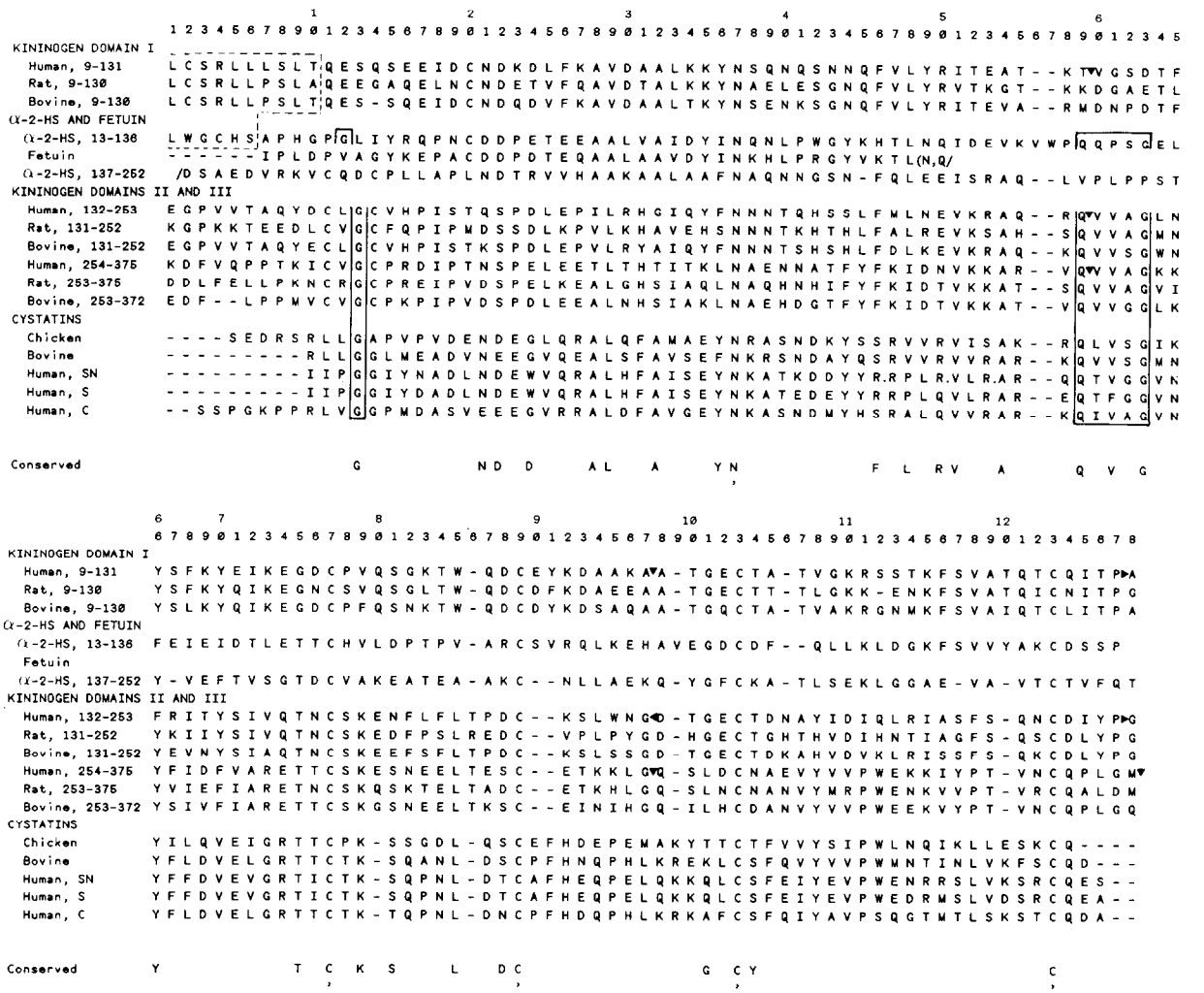

Fig.1. Alignment of cystatins from chicken [19,20], cattle [21], and man [17,22,23] with the cystatin domains of kininogens from cattle [24], man [5], and rat [25] and the cystatin domains of alpha-2-HS-glycoprotein [9,11]. Triangles mark the intron-exon junctions in the human kininogen [7] and their orientation denotes type of junction: ► 1/2, ◄ 2/3, ▼ 3/1; the numbers refer to the positions of adjacent nucleotides within their codons (in the first two cases the triangles point to the residue coded by the spliced codon). Boxed with solid lines are positions probably essential for inhibitory activity [1]. Boxed with a broken line are signal sequences.

ment of fetuin [12] is very similar and evidently homologous to the first cystatin domain of alpha-2-HS. Fetuin is a major plasma protein of fetal ruminants and probably other artiodactyls.

2. RESULTS AND DISCUSSION

A routine search of our Protein Sequence Database using the FASTP program of Lipman and Pearson [13] revealed that alpha-2-HS is similar to the bovine kininogens I and II (with optimized scores of 139 and 140). Comparison of the sequences using our ALIGN program [14] showed that the statistically significant similarity is restricted to the cystatin domains contained in the heavy chain of kininogens. Alpha-2-HS is most similar to bovine kininogen II, with an ALIGN score of 5.9 SD between the corresponding amino-terminal domains and scores of 8.7 and 7.2 SD between the alpha-2-HS second domain and the kininogen second and third domains.

The homology of fetuin and alpha-2-HS is evi-

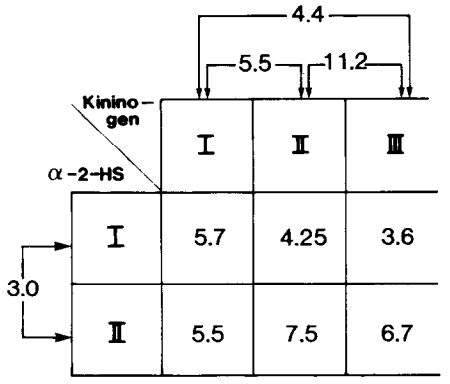

Fig.2. Average ALIGN scores between the cystatin domains in alpha-2-HS-glycoprotein and kininogens.

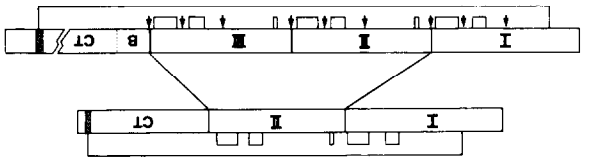

Fig.3. Comparison of the domain structure and disulfide bonding in alpha-2-HS and HMW kininogen. I–III, cystatin domains; CT, non-cystatin, carboxyl-terminal domains; B, bradykinin domain. Arrows mark the intron-exon junctions in kininogen. Except for the carboxyl-terminal domain of kininogen, the domains are drawn to scale.

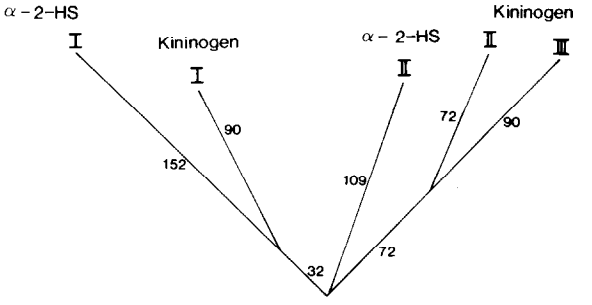

Fig.4. Phylogeny of the cystatin domains generated by the MATTOP tree program described in Hunt et al. [26].

dent (fig.1). The amino-terminal fragment of fetuin is clearly more similar to the first than to the second cystatin domain of alpha-2-HS: 52% of the residues are identical and 9 of these are unique to these two sequences.

The homology of the two amino-terminal domains of alpha-2-HS to the three cystatin domains of kininogens is demonstrated by the alignment (fig.1) and the ALIGN scores (fig.2). Both cystatin domains of alpha-2-HS are more similar to those of kininogens than to cystatins, the similarity to stefins being even more remote. The corresponding domains in alpha-2-HS and kininogen are more similar to each other than are the first and the second domains within each protein (fig.2). The first alpha-2-HS domain scores best with the first kininogen domain, and the second alpha-2-HS domain scores better with either the second or third than with the first kininogen domain. Common to the amino-terminal parts of the second domain of alpha-2-HS and the second and third domain of kininogen are two cysteines, indicative of a small disulfide loop (fig.3), which is unique to these domains (absent in the first domains and cystatins). This parallel pattern of similarity between domains suggests that the genes coding for alpha-2-HS and a protokininogen diverged by duplication of their ancestral gene after the intragenic duplication of the single cystatin domain and before the duplication of the second cystatin domain, as indicated in the computer-derived phylogenetic tree (fig.4).

All cysteines are conserved between the corresponding cystatin domains in the mature kininogen and alpha-2-HS (fig.3). In kininogen [8,15,16] all but the amino-terminal cysteine (alignment position 20) of the mature protein form intradomain disulfide loops and the amino-terminal cysteine bonds with the carboxyl-terminal cysteine in the light chain (Cys-596 in the human mature HMW kininogen). In alpha-2-HS, the two chains are known to be connected by a disulfide bond [11] and, if the intradomain bonds are as in kininogen, the amino-terminal cysteine is the only one remaining to form this bond with the carboxyl-terminal cysteine in chain B. It is therefore very likely that the interchain bonds in alpha-2-HS and kininogen are also homologous (conserved from the common ancestral molecule). The sequences surrounding the carboxyl-terminal cysteines are very similar in alpha-2-HS (PPCPGR) and in the human (PKCPGR) and bovine (PKCPSR) HMW kininogens, but the remaining parts of the carboxyl-terminal, non-cystatin domains show little similarity and that of HMW kininogen is much longer than that of alpha-2-HS. However, the combined evidence from their corresponding carboxyl-terminal positions relative to the homologous cystatin domains, and from the ap-

parent conservation of the interchain disulfide bond, suggests that both proteins inherited a carboxyl, non-cystatin part from their common ancestor. Subsequent evolutionary changes, possibly including incorporation of unrelated segments into one or both molecules, produced dissimilar sequences except surrounding the carboxyl-terminal cysteines.

By circumstantial evidence, the reactive site of cystatin-related inhibitors is expected to involve a string of five residues (QXVXG, predominantly QVVAG) at alignment positions 59–63 (fig.1) [1]. Positions 59–63 are occupied in alpha-2-HS by the sequence QQPSG, which has two residues unknown in other cystatin domains with inhibitory properties. However, the human salivary cystatins, which are strong inhibitors [17], also differ from all others by one (QTVGG in cystatin SN) or two (QTFGG in cystatin S) residues at this site, the replacement of valine by threonine being clearly non-conservative. Also highly conserved throughout the inhibitory domains is glycine at alignment position 13, which in the human cystatin S, cystatin SN, and stefin is preceded by proline. The first domain of alpha-2-HS has a glycine at alignment position 12, which is also preceded by proline.

Delaisse et al. [18] demonstrated that at least three cysteine proteinases, cathepsin B and two probably novel enzymes, participate in the resorption of bone in rodents. In view of the unexplained association of alpha-2-HS with bone tissues, there is a good possibility that its amino-terminal domain acts as a specialized inhibitor of the proteinases responsible for the resorption of bone.

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