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The amino-terminal domain of thrombomodulin and pancreatic stone protein are homologous with lectins

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Amino acid sequence alignment of the amino-terminal domain of thrombomodulin and pancreatic stone protein with the hepatic asialoglycoprotein receptor shows that these proteins are homologous. From the known disulfide bridge pattern of other proteins belonging to the same family two disulfide bonds can be predicted. The homology raises the question whether the amino-terminal part of thrombomodulin and the pancreatic protein binds carbohydrate or perhaps like tetranectin have a specific affinity for other proteins.

Sequence homology; Thrombomodulin; Pancreatic stone protein; Lectin

1. INTRODUCTION

Thrombomodulin is a membrane protein present in the vascular endothelium, where it has an important role as a cofactor in the thrombin catalyzed activation of protein C [1,2]. Complete human cDNA [3-5] and partial bovine cDNA [6] sequences have been determined, showing that the protein can be organized into an amino-terminal domain followed by six repeats of the epidermal growth factor type, a stretch rich in potentially *O*-glycosylation sites, a membrane spanning segment, and finally a cytoplasmic domain. Although a weak similarity with mullerian inhibiting substance was identified [5], no clear homology between other proteins and the amino-terminal domain was detected by standard computer programs [3-5].

Pancreatic stone protein [7], also called pancreatic thread protein [8], is a calcium binding protein found in the pancreatic juice. The protein or fragments of the protein have a tendency to form fibrils [9], and it inhibits precipitation of calcium carbonate [10].

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In this paper I have aligned the amino-terminal part of thrombomodulin and the pancreatic stone protein with the family of lectins represented by the chicken hepatic asialoglycoprotein receptor [11].

2. METHODS

The GENEPRO software system (version 4.1) for handling protein sequences including the PIR database version 12 from the National Biomedical Research Foundation was obtained from Riverside Scientific Enterprises, Seattle WA, USA. The programs were installed on an IBM AT personal computer. Searching for sequence similarities was performed with a window of 25 residues out of which 9 residues should be identical before a similarity was recognized. The most promising similarities, as judged by the mean log-odds score and biochemical soundness, were further analyzed by a dot matrix plot.

3. RESULTS AND DISCUSSION

The family of proteins showing homology with the hepatic receptor that recognizes the carbohydrate of desialated plasma proteins [11-13] is rapidly growing. This group now includes mannose-binding proteins [14,15], lung surfactant protein [14,16], cartilage proteoglycans [17,18], tetranectin, which is a protein from plasma that binds to kringle 4 of plasminogen [19], and the

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lymphocyte receptor for immunoglobulin E [20]. Proteins from invertebrates such as echinoidin from the sea urchin [21], and lectins from acorn barnacle [22] and the larvae of a flesh-fly *Sarcophaga peregrina* [23] also belong to the family.

In fig.1 the amino-terminal 154 residues of thrombomodulin and the pancreatic stone protein are aligned with selected members of the asialoglycoprotein family. The amount of similarity between thrombomodulin and the proteins in fig.1 is 15–20% calculated as the number of identical residues relative to the total number in the align-

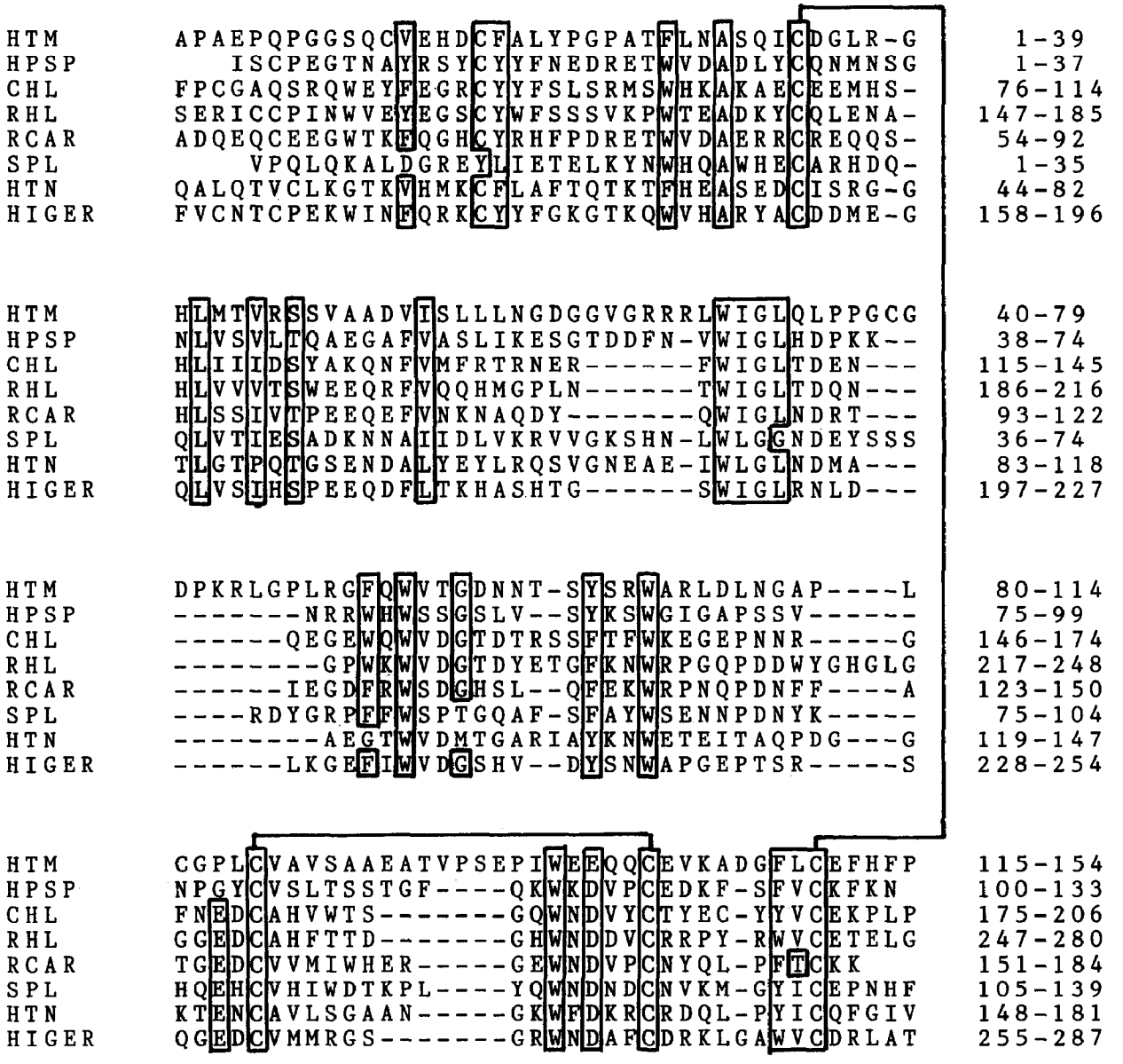

Fig.1. Alignment of the amino-terminal part of thrombomodulin HTM [3–5] and human pancreatic stone protein HPSP [7] with selected proteins of the lectin family. Chicken hepatic lectin CHL [11], rat hepatic lectin RHL [12], rat cartilage proteoglycan RCAR [17], flesh-fly *Sarcophaga peregrina* lectin SPL [23], human tetranectin HTN [19] and human IgE receptor HIGER [20]. Areas with a high degree of conservation are boxed. Numbers refers to positions in the mature peptide chain. The pancreatic stone protein is also found with an extension at the amino-terminal side suggesting that the present sequence is a result of limited proteolysis [24]. The number for the rat cartilage proteoglycan refers to the partially known cDNA [17].

ment. The 20% similarity is with the human pancreatic stone protein and the IgE receptor. The similarity is slightly higher (22%) with the partially known sequence of bovine pancreatic protein [8] (not included in fig.1). Two insertions have to be introduced in the thrombomodulin sequence corresponding to residues 78–85 and 129–132, but the other proteins also show some variation in that part of their sequence.

Four half-cystines are conserved in all the proteins corresponding to residues 34, 119, 140 and 149 in thrombomodulin. The disulfide pattern has been determined for the half-cystines in the acorn barnacle [22] and sea urchin lectins [21] as well as tetranectin [19] predicting the disulfide bonds Cys-34–Cys-149 and Cys-119–Cys-140 in thrombomodulin. The alignment with lectins ends at approximately residue 155, leaving 70 residues in front of the first half-cystine in the first epidermal growth factor-like repeat. This part has a relatively high degree of hydrophobic amino acids and could constitute a structural domain in its own.

The pancreatic protein shows a greater similarity with the proteins in fig.1 varying from 19–31%. The 31% similarity is with the rat cartilage proteoglycan and the IgE receptor. The protein contains six half-cystines of which one bridge has been identified between Cys-3 and Cys-14 [24]. Based on the homology, two other bridges can be predicted as Cys-31–Cys-128 and Cys-104–Cys-121.

The present alignment does not necessarily mean that thrombomodulin and the pancreatic stone protein are lectins themselves. Tetranectin for example, was identified and isolated [25] by its specific binding to kringle 4 of plasminogen which does not contain any carbohydrate [26].

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