

Homology with hemopexin suggests a possible scavenging function for S-protein/vitronectin

K.K. Stanley

European Molecular Biology Laboratory, Postfach 10.2209, Meyerhofstrasse 1, 6900 Heidelberg, FRG

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S-protein is an abundant plasma protein which has recently been shown to be identical to vitronectin and serum spreading factor [(1985) EMBO J. 4, 3153–3157]. It therefore has multiple binding sites for terminal complement complexes, thrombin-antithrombin III, heparin, and a specific cell receptor. In this report a structural and sequence homology with hemopexin is described which suggests that the principle function of S-protein could be as a scavenging molecule, clearing spent complement and coagulation complexes from the circulation

S-protein Vitronectin Serum-spreading factor Hemopexin Sequence homology Structural homology

1. INTRODUCTION

S-protein, vitronectin and serum spreading factor all refer to the same 75 kDa protein of plasma, the different names reflecting the ways in which the protein was discovered. S-protein stands for 'complement membrane-site equivalent' [2] and is derived from the ability of S-protein to bind to nascent terminal complement complexes generated in the fluid phase [2–5], thus preventing membrane integration and protecting bystander cells from lysis [2–4,6]. The same protein was isolated from serum by its ability to bind to glass beads [7] and was therefore called vitronectin [8,9]. Binding studies showed that the protein had fragments capable of binding to heparin [10] and cell surface receptors [11,12], and when coated on plastic or glass surfaces has a cell-spreading activity [13]. These studies suggested that vitronectin/serum spreading factor might function as a cell adhesion molecule of the extracellular matrix, although its abundance in plasma is not explained on this hypothesis. S-protein also forms stable complexes with thrombin-antithrombin III [14,15], suggesting a further possible role in the coagulation pathway. The homology reported here between S-

protein and hemopexin raises the possibility that S-protein functions as a scavenging molecule for inactive terminal complement and coagulation complexes. This hypothesis can account for its multiple binding activities and high concentration in plasma.

2. RESULTS AND DISCUSSION

Comparison of the S-protein amino acid sequence with the protein sequence data library of the National Biomedical Research Foundation using the algorithm developed by Lipman and Pearson [16] suggested a homology between the central portion of S-protein and hemopexin. This homology had presumably not been noticed previously because 3 bases missing in the vitronectin cDNA sequence [1,12] cause a reading frame shift in one of the best regions of homology. Published comparisons of the hemopexin sequence with itself have suggested that the two halves of the protein are related [17,18] and that 5 structurally related segments can be discerned in each half of the molecule [18]. Comparisons of S-protein with itself and with hemopexin show that S-protein also

contains 10 repeats although, like hemopexin [18], some of these repeats are closer to the consensus than others.

Fig.1 shows the internal homology found by comparing the sequence of S-protein with itself on the basis of residue physical parameters [19] which are important for protein folding. All values at each residue position along one axis of the matrix comparison table have been summed and smoothed according to the method of Argos [18]. This method emphasises repeating structures and gives an estimate of the sequence coordinates of each repeat. Six major peaks can be seen in this figure (numbered 3–7, 10), each followed by a subsidiary peak arising as a result of homology being found more strongly within the second half of some repeats. In addition, minor peaks can be seen before peak 3, and in the gap between peaks 7 and 10, suggesting that weakly related sequences may be present in the other 4 positions giving a total of 10 repeats as is found in hemopexin. All of the major self-homologous segments of the S-protein sequence show structural homology with most of the hemopexin repeats (fig.2). No overall homology could be discerned, however (which would give rise to a continuous diagonal line from bottom left to top right), suggesting that considerable divergence of the two proteins must have occurred.

Using the combined information of figs 1 and 2 it was possible to determine the precise starting positions of the 6 major homologous regions of S-protein. These regions shared the same consensus sequence as that already determined for hemopexin [18], having a conserved glycine residue in a hydrophobic environment at the amino-terminal end, and an aspartic acid followed by two alanine residues at the carboxy-terminal end (marked with an asterisk in fig.3). The remaining 4 regions corresponded to the somatomedin B domain, the heparin-binding site, and two other regions without described functional properties. In each of these regions sequences approximating the consensus sequence could be found by visual inspection and are included in fig.3. These sequences give rise to the small peaks of self-homology in fig.1. It is interesting to note that the somatomedin B and heparin-binding site occupy exactly one repeat in this alignment, and also that the cell attachment site (box, fig.3) and two known cleavage points of the molecule (arrows, fig.3), which are likely to be

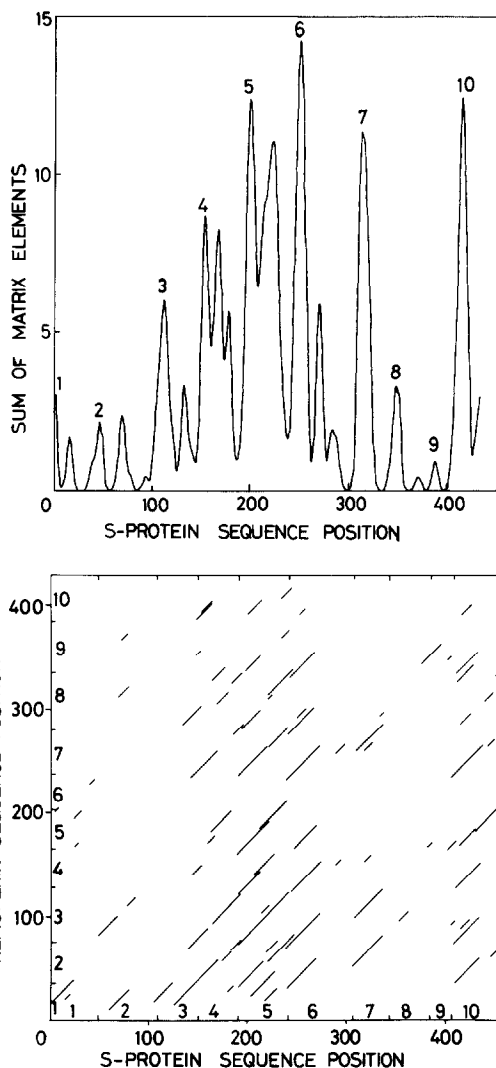


Fig.1(Top). Self-homology of S-protein determined by scoring all possible comparisons of sequence spans 15 residues in length on the basis of 6 physical parameters important for protein folding [30] and the Dayhoff relatedness odds matrix [31–33]. Values greater than 3.5 SD above the mean of the comparison matrix were summed at each sequence position as described [18]. The peaks corresponding to the aligned sequences in fig.3 are numbered 1–10.

Fig.2(Bottom). Predicted structural homology between S-protein and hemopexin. The two sequences were compared as described in fig.1 a total of 16 times using windows of comparison between 5 and 20 residues in length. Values in each comparison matrix which were 4 SD or more above the mean matrix value are displayed as diagonal lines the length of the respective windows. Divisions marked inside the axes show the starting residues of the aligned sequences in fig.3.

Starting No. Residue		S-PROTEIN	
1	1	-----DQESCKGRCTEGF-----	NVDKKQCDELCSYYQSCCTDYTAECKPQVT RGD VFT
2	51	---MPEDEYTVYDDPGEEK-----	---NNATVHEQVGGPSLTSDLQAQSKGNPEQT TPVLK
3	100	EEEAPAPEVGASKPEGIDSR-----	PETLHPGRPQPPAEEELCS-G--KPFDAFTDLK-----
4	150	-----NGSLFAFRGQYCY-----	ELDEKAVRPGYPKLI RDVW-GIEGPIDAAF-----
5	192	--TRINCQGKTYLFKGNQYWRF-----	EDGVLDPDYPRNISDGFDFGIPDNVDAALAL-----
6	242	PAHSYSGRERVYFFKGKQYW---EYQFQHQP	SECEGSSLSAVFEHFAMMQRDSWEDIFEL-----
7	301	---LFWGRTSAGTRQP-----	---QFISRDWHGVPQGVDAAMAGRIYISGMAPRP
8	345	-SLTKKQRFRRNRKGYRSQ-----	RGHSRGRNQNSRRPSRAMWL-----
9	384	---SLFSSEESNLGANNY-----	---DDY-----RMDWLVPA-----
10	410	---TCEPIQSVFFFSGDKYYRV-----	NLRTRRVDTVDPYPYRSIAQYWLGCAPAGHL-----
		HAEMOPEXIN	
1	1	-----TLPPTSAGHNVAE-----	GETKPPDPVTERCSDGW--SFDATT-----
2	38	---LDDNGTMLFFKGEFVW-----	KSHKWDRELISERWKNFSPVDAAF-----
3	79	---RQGHNSVFLIKGDKVWVY-----	PPEKKEKGYPKLLQDEFPGIPSPLDAAVECHRGEC-----
4	132	---QAEGVLFFQGDREWFV-----	DLATGTMKERSWPAVGN-CSSAL-----
5	170	---RWLGRIYCFQGNQFLRF-----	DPVRGEVPPRYPR--DVRDYFMPCP-----
6	210	---GRGHGHRNGTGHGNS-----	THHGPEYM-RCSPHL--VLSALT-----
7	245	---SDNHGATYAFSGTHYWRL-----	DTSRDGWHSWPIAHQWPQGPSAVDAAF-----
8	290	---SWECKLYLVQGTQVYVFLTKGGYTLVSGYPKRLEKEVGTPHGIILDSVDAAFICP-----	
9	345	---GSSRLHIMAGRRLLWWL-----	DLKSGAQTATWTELPWPHEKVDGALCMEKSLGPNSC-----
10	396	---SANGPGLYLIHGPNLYCY-----	SDVEKLNAAKALPQPN-VTSLLGCTH-----
		++++ * ++	++ +***+

Fig.3. Aligned repeat unit in S-protein and hemopexin. The 10 related sequences of S-protein and hemopexin are shown aligned by the conserved Gly and Asp residues at either end of the repeat. Exposed cleavage sites in S-protein are indicated with arrows, the cell attachment site by a box, and putative sites for *N*-linked oligosaccharide attachment are shown underlined. Repeat 1 of the S-protein is the somatomedin B fragment, and repeat 8 the heparin-binding site. Amino acids occurring 10 or more times in the same position are shown under the aligned sequences with an asterisk, those of conserved character according to the scheme: (P,G); (T,S); (K,R); (D,E,Q,N); (A,V,I,C,M,L,H,F,Y,N); are shown by a +.

exposed regions, lie in the same relative position in the repeat.

Despite the evidence for a duplication of sequence in hemopexin only two of its disulphide bonds are conserved [17]. These connect a cysteine in the first and sixth repeat of the molecule (residues 27 and 234) to one in the fifth and tenth repeat (residues 208 and 433). In S-protein the carboxy-terminal fragment cleaved by trypsin at residue 380 [1,20] is not released unless the molecule is reduced [14,20-22], indicating that it is held by a disulphide bond. One of the 2 cysteine residues in this fragment lies in the tenth repeat of S-protein and in the same relative position as cysteines 208 and 433 of hemopexin. It will be interesting to determine if this cysteine is connected across the second half of the molecule to the cysteine residue in the sixth repeat. There is unfortunately no obvious similarity between the position

of the cell binding and heparin-binding sites of S-protein and histidine residues of hemopexin which might be involved in heme binding.

S-protein and hemopexin are related in several ways. Both are glycoproteins of similar *M_r*, synthesised in the liver, which are present at high concentration in plasma. Both molecules form stable complexes with inactive plasma proteins which have to be cleared from the circulation. Both have cell binding sites, although only S-protein has the Arg-Gly-Asp tripeptide common to fibrinogen, fibronectin, and Von Willebrand factor [11,23,24]. Finally, both proteins can undergo conformational changes to expose new binding sites, for a cell surface receptor in the case of hemopexin [25,26], and for heparin-like molecules in the case of S-protein [27]. Heparin binding could account for the S-protein interaction with thrombin-antithrombin III, since excess heparin inhibits S-protein binding

in this reaction [15], and excess S-protein abolishes the heparin stimulation of antithrombin [21]. Little is known about the interaction of terminal complement complexes with S-protein, both hydrophobic [4,6] and hydrophilic [14] interactions having been proposed. However, it is possible that complement component C9 could present a heparin-like site since it contains a highly negatively charged cysteine-rich domain bearing homology to the LDL receptor [28] which is known to bind its ligand in a way which is competitively inhibited by heparin [29].

The homology reported here now suggests that both molecules are members of the same gene family. These similarities raise the possibility that S-protein might have a scavenging role, like that of hemopexin, but specific for spent complexes of the complement and coagulation pathways. In this way the multiple binding properties of the molecule can be accounted for. The previously described cell spreading activity of the protein may simply be a result of the assay system used, glass and plastic surfaces exposing the cell attachment site(s) in a similar manner to terminal complement and thrombin-antithrombin III complexes *in vivo*. Experiments are presently in progress to test this hypothesis.

S-protein has been given a number of names according to how it was first described (S-protein, vitronectin, serum spreading factor, and protein-X [15,22]). Now that its genealogy has been found perhaps it should be renamed 'heparopexin' according to its family name (pexin) and its principle ligand (heparin-like molecules).

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