Vitamin K-dependent protein S is similar to rat androgen-binding protein

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Vitamin K-dependent protein S belongs to the family of clotting factors (e.g. Factors IX and X, and protein C). Unlike the other clotting factors, the C-terminal half (residues 250–634) of protein S is not a serine proteinase. In fact, the function of residues 250–634 of protein S is unknown. By using computer programs designed to detect evolutionary relationships between proteins, we find that this part of protein S is similar to rat androgen-binding protein, a protein produced and secreted by testicular Sertoli cells. The homology between protein S and androgen-binding protein suggests new approaches for elucidating their functions.

INTRODUCTION

Protein S is a vitamin K-dependent protein (DiScipio & Davie, 1979) that has at least two physiological properties: (1) it is a cofactor with activated protein C in the regulation of blood clotting (Walker, 1981; Gardiner *et al.*, 1984), and (2) it forms a non-covalent complex with the regulatory complement-protein-C4b-binding protein (Dahlbäck & Stenflo, 1981; Dahlbäck, 1983). People with a hereditary protein S deficiency are predisposed towards venous thrombosis (Schwarz *et al.*, 1984; Comp *et al.*, 1984). Despite the physiological importance of protein S, most of the details of its biological actions remain unknown. The determination of the primary amino acid sequence of protein S was an important step towards unravelling the functions of this protein (Dahlbäck *et al.*, 1986). The amino acid sequence revealed that the first approx. 250 residues from the N-terminus of protein S are similar in structure to other vitamin K-dependent clotting factors. First there is a 44-residue domain which contains the γ -carboxyglutamic acid (Gla) residues. After this sequence is a 30-residue segment that is sensitive to thrombin, which is followed by a 160-residue segment that is similar to epidermal growth factor (Dahlbäck *et al.*, 1986). The function of the remainder of the C-terminal sequence, residues 250–634, which constitutes about 60% of protein S, is unknown. It is clear, however, that it does not have any similarity to serine proteinases, which is what might be expected by analogy to other clotting factors (Patthy, 1985). Serendipitously, as part of a project to search for similarities between steroid-hormone-binding proteins and serine proteinases, one of us (Baker, 1985) discovered a strong similarity between the amino acid sequences of this C-terminal domain of protein S and that of rat androgen-binding protein (ABP). ABP is produced and secreted by testicular Sertoli cells and binds androgens and oestrogens with high affinity (French & Ritzen, 1973; Hansson *et al.*, 1973, 1976). The homology between ABP and protein S suggests an interesting genealogy for protein S and the possibility for novel ways for regulating the clotting process.

RESULTS AND DISCUSSION

The comparisons of the sequences of protein S (Dahlbäck *et al.*, 1986) and ABP (Joseph *et al.*, 1986) were done by using the RELATE and ALIGN computer programs, which were developed at the National Biomedical Research Foundation to detect evolutionary relationships between proteins (Barker & Dayhoff, 1982; Dayhoff *et al.*, 1983). A RELATE analysis of protein S with ABP using a segment length of 30 gave a comparison score that was 17.4 s.d. higher than that of 100 comparisons of randomized sequences of these proteins. The probability of getting this score by chance is less than 10^{-50} . Fig. 1 is a graphical representation (Maizel & Lenk, 1981; Staden, 1982) of the similarity between residues 250–634 of protein S and ABP. Similar segments extend throughout ABP and the C-terminus part of protein S, and there is an off-diagonal similarity between residues 221–254 of ABP and residues 282–315 of protein S. The segments along the diagonal of Fig. 1 are presented in more detail in Fig. 2. An ALIGN analysis of segments A, C, and D of protein S and ABP gave comparison scores that were 13.5, 10.5 and 6.75 s.d. higher respectively than that obtained with 2500 comparisons of randomized sequences of these proteins. The probability of getting these scores by chance is less than 10^{-40} , 10^{-25} and 10^{-11} respectively. An ALIGN analysis of residues 221–254 of ABP with residues 282–315 of protein S yields a score of 7.4 s.d. units. Recently, the amino acid sequence of human sex-steroid-binding protein (SBP) was determined (Walsh *et al.*, 1986), and it was found to be 70% homologous with that of rat ABP (Joseph *et al.*, 1986; Walsh *et al.*, 1986). The RELATE comparison of SBP with protein S yields a comparison score 16.7 s.d. higher than that of 100 comparisons of randomized sequences of these proteins, and the distribution of similar segments between these proteins and their ALIGN comparison scores are like that of ABP and protein S.

The vitamin K-dependent clotting factors are an example of the shuffling of exons or modules with different functions to assemble proteins to meet a variety

Fig. 1. Graphical representation of the similarity between bovine protein S and rat ABP (Maizel & Lenk, 1981; Staden, 1982)

ABP and residues 245–624 of protein S were compared by using a 20-residue segment. Each pair of residues on these proteins was scored using the Mutation Data Matrix (Barker & Dayhoff, 1982; Dayhoff *et al.*, 1983). Segments with a score of 20 or larger are represented by a point at the midpoint of the segment. The segments along the diagonal are described in more detail in Fig. 2. Residues 282–315 of protein S appear to be similar to a second segment on ABP, residues 221–254. Disulphide bridges between residues 408 and 434, and 597 and 624 of protein S and residues 164 and 188, and 333 and 361 of ABP are shown as proposed by Dählback *et al.* (1986) and Walsh *et al.* (1986) respectively.

of biological needs (Doolittle, 1985; Patthy, 1985). All members of this family contain a vitamin K-modified domain, which is Gla-rich, at their *N*-terminus. This

domain can bind Ca^{2+} tightly. In protein S this domain is important for its binding to phospholipid membrane surfaces, which is important in its cofactor activity with activated protein C. In protein S, Factors IX and X, and proteins C and Z, this Gla-rich segment is followed by a domain that is called the 'growth-factor domain' because its amino acid sequence is similar to that of part of epidermal growth factor (Young *et al.*, 1978; Doolittle *et al.*, 1984; Doolittle, 1985; Patthy, 1985; Dählback *et al.*, 1986). An explicit function for this domain has not yet been established. In thrombin, another vitamin K-dependent protein, the Gla-rich domain is followed by two kringle structures, and there are no growth-factor domains. The last module of the vitamin K-dependent proteins, which comprises the rest of the *C*-terminal sequence, is a serine proteinase, or in the case of protein Z, a non-catalytically active domain, that is clearly homologous with serine proteinases (Hojrup *et al.*, 1985). The fact that protein S lacks this serine-proteinase-like module makes it unique among the vitamin K-dependent clotting factors.

The computer-based analysis presented here clearly show that this latter module of protein S is homologous with ABP (Figs. 1 and 2). The similarities in the sequences extend over most of ABP and residues 250–634 of protein S. Important for a possible functional similarity between these proteins is the apparent conservation of four cysteine residues and their disulphide linkages on protein S and ABP. Moreover, the gaps between the homologous segments are sufficiently similar in size to permit the segments to align in the tertiary structure of these proteins. Interestingly, segment A (Fig. 2), where the greatest conservation of the sequences of protein S and ABP occurs, is also the region where there is 90% homology between ABP and SBP (Joseph *et al.*, 1986; Walsh *et al.*, 1986), which suggests an important functional role for this segment in protein S, ABP and SBP. All of the above suggests that this last module on protein S is likely to have some biological properties in common with ABP, which at first glance

(A) Protein S 269 V L Y L K F R L P E T T R F S A E F D F R T Y D S E G V I L Y A E S S D H S A W F
ABP 27 V T V L T I R D L T K I S K P S S S F E F R T W D P E G V I F Y G D T N T E D D W F

Protein S 310 L I A L R E G K I E I Q F K N E K T T K M T T G G K V I N D G L W H M V S V 347
ABP 68 M L G L R D G Q L E I Q L H N L W A R L T V G F G P R L N D G R W H P V E L 105

(B) Protein S 400 P I N P R L D G C I R 410
ABP 156 P L V P A L D G C I R 166

(C) Protein S 433 H C L V N V E K G S Y Y P G T G V A Q F S I N Y K N E S N P E A W Q I N V S L
ABP 187 N C D V D L Q P G L F F P P G T H A E F S L Q D I P Q P H T D P W T F S E L

Protein S 472 N I R P S A G T G V M L A L 486
ABP 226 G F K L V D G A G R L L T L 240

(D) Protein S 597 C M E V N I N G V Q V D L D E A I S K H N D I R A H S C P S V W Q K T K H T 634
ABP 333 C L S D L W V Q G Q R L D L D K A L S R S Q D I W T H S C P Q S P S N D T H T 371

Fig. 2. Comparison of bovine protein S with Rat ABP

Continuous-line boxes show identities and the broken-line boxes show conservative replacements according to the scheme: (P,G), (M,C), (Y,W,F,H), (L,V,I,A), (K,R), (E,Q,N,D) and (S,T). Cysteine-164, -188, -333 and -361 of ABP align with cysteine-408, 434, -597 and -624 respectively of protein S. The one-letter amino acid notation is used.

would be expected to be binding a hydrophobic ligand. Because protein S has at least two protein-binding activities, which may involve residues 250–634, the possibility that hydrophobic ligands have a role in regulating these actions of protein S needs to be considered.

Even though ABP and SBP bind androgens and oestrogens with high affinity, the function(s) of this and their other properties (Hryb *et al.*, 1985; Martin *et al.*, 1986) are still obscure. The similarities between protein S and ABP and SBP suggest that, as the properties of residues 250–634 of protein S are deciphered, new activities for ABP and SBP may be revealed.

The computer analyses cannot resolve the question of the origin of residues 250–634 of protein S. It is clear, however, that the first 250 residues of protein S are related to those of the other clotting factors. The absence of a serine-proteinase domain on protein S led Dahlbäck *et al.* (1986) to place protein S on branch separate from a branch containing thrombin, a kringle-containing clotting factor. The genealogy of the clotting factors is enriched by their similarity to members of the fibrinolytic factors, which contain growth-factor domains, kringles, and are serine proteinases (Young *et al.*, 1978; Doolittle, 1985; Patthy, 1985). The shuffling of different functional modules among all of these proteins is one of the best examples of the variation of a few well-defined functions to achieve the diversity that is needed for the coagulation and fibrinolytic processes. Protein S, however, is unusual among these proteins in that the module that would be expected to be a serine proteinase is instead similar to a steroid-binding protein. We consider two possibilities for the origin of this module on protein S. First, it could be derived from a gene coding for a steroid-binding protein, which fused with the Gla-rich domain and growth-factor domain of a clotting-factor ancestor.

Second, it could be derived from the serine-proteinase ancestor that likely is common to the clotting and fibrinolytic factors. In this case, a combination of amino acid substitutions, frameshift mutations and gene duplications obscure the evidence in the primary amino acid sequence for its origin. The similarity between residues 282–315 of protein S and two segments of ABP suggests that a gene duplication has occurred in these proteins, which could obscure their origin. Also relevant to this possibility is the extensive divergence in the primary amino acid sequences of the mammalian pancreatic and bacterial serine proteinases, which makes their sequence similarity marginal at best (James *et al.*, 1978; Young *et al.*, 1978; McLachlan, 1979; Doolittle, 1981), because a comparison of the tertiary structure of these enzymes, which is a more sensitive technique, clearly shows their common ancestry (James *et al.*, 1978; McLachlan, 1979). The determination of the tertiary structure of protein S, ABP or SBP would more definitively resolve the ancestry of these proteins. Interestingly, there is some evidence for chemical similarities between steroid-hormone-binding proteins, including ABP and SBP, and proteinases. Serine-proteinase inhibitors (e.g. di-isopropyl fluorophosphate or tosylphenylalanylchloromethane) and substrates (e.g. tryptophan methyl ester or tosylphenylalanine methyl ester) can inhibit the binding of steroids to SBP (M. E. Baker, unpublished work), rat α -foetoprotein and intracellular receptors (Hunziker *et al.*, 1980; Lukola &

Punnonen, 1982; Baker, 1985, 1986; Baker *et al.*, 1985; Hubbard & Kalimi, 1985; Puca *et al.*, 1986). One explanation for these findings is that the tertiary structure of steroid-binding proteins has some chemistry in common with proteinases (Baker, 1985, 1986; Baker *et al.*, 1985).

Whatever the ancestry of protein S and ABP, the similarities in their amino acid sequences suggests that, as each of these proteins becomes better understood, the resulting information could provide clues for understanding the functions of the other.

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