AMINO ACID SEQUENCE OF A PEPTIDE FROM BOVINE PLASMA FIBRONECTIN CONTAINING A FREE SULFHYDRYL GROUP (CYSTEINE)

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Received 13 April 1982

1. Introduction

Plasma fibronectin is a glycoprotein which consists of 2 disulphide-bridged polypeptide chains of M_r 215 000 and 220 000, respectively [1,2], whereas fibronectin associated with the cell surface is found both as a dimer and as a multimer [3]. Fibronectin forms strong non-covalent bonds with collagen [4] and heparin [5]. It interacts with cell surfaces [6] and has been reported to bind some other macromolecules including actin [7] and fibrin [8]. Fibronectin can be covalently crosslinked by plasma transglutaminase (factor $XIII_a$) [9] to fibrin [10], collagen [11], *Staphylococcus aureus* cells [12] and to itself [13]. Cellular fibronectin agglutinates sheep erythrocytes [14] and restores normal fibroblastic morphology to transformed cells [14]. Plasma fibronectin is necessary for opsonization of gelatin-coated particles [15].

Degradation of fibronectin with chemicals or proteolytic enzymes has revealed a domain structure (reviewed in [16]) with different domains taking part in crosslinking by factor $XIII_a$ [10,17,18], binding to cells [19], collagen [20] and heparin [19,21]. Fibronectin contains 1–2 thiol groups/monomer [16,22–25], and blockage of the free sulfhydryl group prevents binding to cells [22]. Cleavage by *S*-cyanylation [25] suggests a cysteine located 170 000 mass units from the N-terminus.

After digestion of bovine fibronectin with plasmin, 4 fragments of M_r 29 000 (29k), 170 000 (170k), 23 000 (23k) and 6000 (6k) have been isolated [26] and here we report the amino acid sequence of a stretch of 27 amino acid residues of bovine fibronectin containing a cysteine, labelled by treatment of denatured 170k with ^{14}C-labelled iodoacetic acid.

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2. Experimental

Bovine plasma fibronectin was purified essentially as described for the human protein [4] by batch adsorption to gelatin–agarose followed by elution with a gradient of urea. The fractions containing fibronectin were pooled and dialysed against demineralised water, and 0.05 M NH_4HCO_3 before digestion with plasmin. Human plasminogen was purified as in [27] and activated with streptokinase (Kabi). Elastase was a gift from D. Shotton. Peptides were sequenced on a Beckman 890 sequencer, using polyprene [28] and the phenylisothiocyanate derivatives were identified by high-performance liquid chromatography [29]. For all other experimental methods, see [30].

Fibronectin (4 g) was digested with 40 mg activated plasminogen and the fragments obtained were purified on columns of DEAE-cellulose and gelatin–agarose as in [17]. The effluent was monitored for thiol groups by reacting aliquots (0.2–0.6 ml) with 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) (Fluka) in the presence or absence of 6 M guanidine–HCl [31]. The fractions showing positive reaction were collected in one pool, dialysed against demineralised water, lyophilised and redissolved in 50 ml 6 M guanidine–HCl, 0.2 M Tris, 0.01 M EDTA (pH 8.3). The solution was incubated with 150 μCi 2-[^{14}C]iodoacetic acid (54 Ci/mol, Amersham) for 1 h and then with a surplus of unlabelled sodium iodoacetate. The reaction mixture was dialyzed against running tap water, followed by demineralized water and after lyophilisation, reduced and alkylated with unlabelled sodium iodoacetate as in [30]. Following resalting on Sephadex G-25 in

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00145793/82/0000–0000/$02.75

0.1 M NH_4HCO_3 the protein solution was incubated with 76 mg trypsin (Worthington) for 20 h at 37°C. The digestion was stopped by adding soybean trypsin inhibitor. The digestion mixture was fractionated on a column of Sephacryl S-200 in 0.1 M NH_4HCO_3 (pH 8.3). The radioactive fractions were collected in one pool and further separated on a column of DEAE-Sephacel (1 × 25 cm) in a linear gradient from 0.1–0.5 M NH_4HCO_3 (2 × 250 ml).

3. Results

Of the 4 main fragments 29k, 170k, 23k and 6k, obtained by digestion of bovine plasma fibronectin with plasmin [26] only 170k showed a positive reaction with DTNB. After labelling of this fragment with radioactive iodoacetic acid under denaturing conditions, the fragment was reduced, alkylated and digested with trypsin. The obtained peptides were fractionated by gel filtration and ion-exchange chromatography and the eluted radioactivity was found in one peak, called T1. A sample of T1 was further digested with chymotrypsin and the peptides were purified by high-voltage paper electrophoresis and paper chromatography. Only one radioactive peptide was found, corresponding to T1C1 in fig.1. When T1C1 was sequenced, a radioactive carboxymethylcysteine was identified in position 13. In order to obtain more sequence information on the C-terminal site of the cysteine another sample of T1 was digested with elastase and after high-voltage paper electrophoresis at pH 6.5 and 1.9 a radioactive peptide was isolated, corresponding to T1E1 in fig.1. In the sequence of T1E1 a clear radioactive carboxymethylcysteine was identified in step two, corresponding to position 13 on fig.1. The sequences of T1C1 and T1E1 are in accordance with their amino acid compositions.

The peptide T1E1 was neutral at pH 1.9 and the amino acid analysis showed that it contained glucosamine after hydrolysis. To localize the attachment site for the oligosaccharide group a sample of T1E1 was digested with the *S. aureus* protease and the peptides T1E1Sp1, T1E1Sp2 and T1E1Sp3 were isolated by high-voltage paper electrophoresis and analysed. They were identified by amino acid composition and electrophoretic mobility. Only T1E1Sp3 was found to contain glucosamine after hydrolysis. Sequence determination of T1E1Sp3 by the dansyl-Edman technique identified an Asx-residue in step two, corresponding to the Asn-25 in fig.1. As the peptide contains only one Asx, this residue must be the attachment site for the carbohydrate. When sequencing T1E1 no phenylisothiocyanate derivative was identified in the corresponding position. Although Asn-17 is in an Asn–X–Ser sequence, no evidence was found for carbohydrate in this position.

Another sample of T1E1 was digested with chymotrypsin and the amino acid compositions and electrophoretic mobilities of the purified peptides (T1E1C1–C3) further confirmed the sequence of T1E1.

4. Discussion

Three different types (I–III) of internal homology have been found in bovine fibronectin [32] and from the sequence of 3 CNBr fragments absent in half-cysteines and carbohydrate, 3 domains of homology type III have been identified (P. S., unpublished). Parts of these domains are shown in fig.2 aligned with

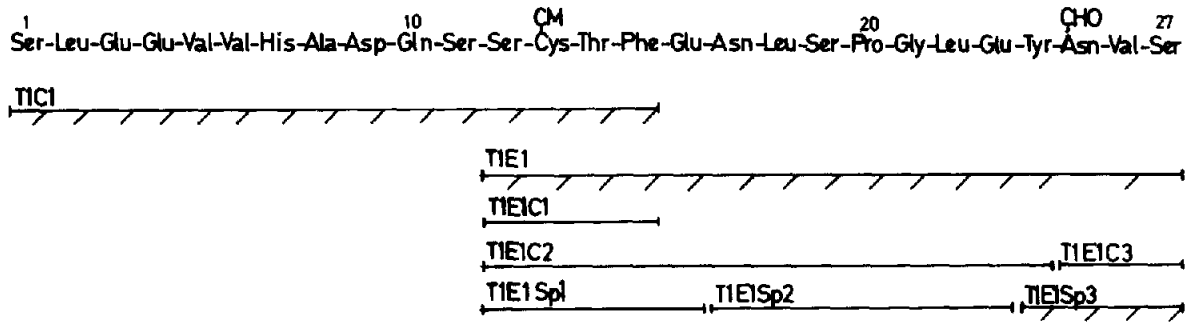

Fig.1. The sequence of a cysteine containing region of bovine plasma fibronectin; CHO, carbohydrate. The peptides were obtained by digestion with trypsin (T), chymotrypsin (C), elastase (E) or *S. aureus* protease (Sp).

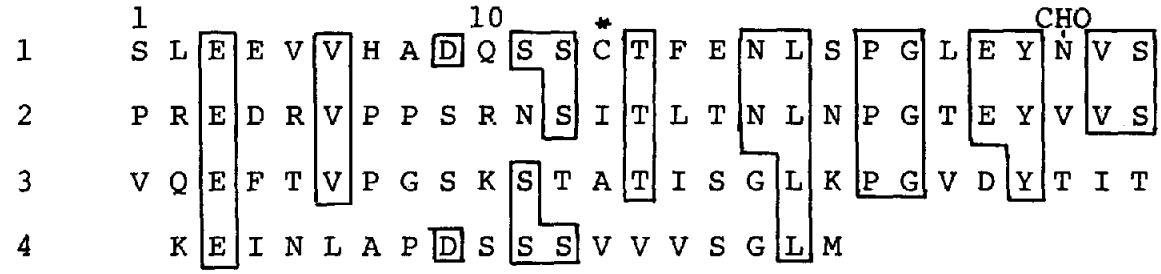

Fig.2. Alignment of sequences from bovine fibronectin (homology type III): (1) cysteine containing region (this paper); (2,3) sequences from a CNBr-fragment of 170k [32]; (4) a CNBr-fragment of 170k, which has also been found in a heparin binding fragment [32]; CHO, carbohydrate; C*, radioactive carboxymethylcysteine.

the sequence from fig.1, clearly showing that this cysteine containing sequence originates from a homology type III domain. Whether there is one or more cysteine residues/chain in bovine plasma fibronectin cannot be answered from the present experiments, but no indications for more than the cysteine shown in fig.1 have been obtained.

The proximity between the cysteine and a site with carbohydrate and a potential site without carbohydrate (Asn–Leu–Ser, residues 17–19), are similar to the situation in the human complement factor B. In this protein a site with carbohydrate has been found 10 residues before a cysteine and an Asn–Leu–Thr sequence without carbohydrate 3 residues after the cysteine [33]. Whether this is a coincidence remains to be seen.

Acknowledgements

Margit S. Jensen has assisted in the purification of fibronectin. This work was supported by the Danish Science Research Council grant 11-3167, and the US National Heart, Lung and Blood Institute, NIH, Bethesda MD, grant HL 16238.

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