Vol. 99, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 16, 1981 Pages 30-36

NH_2-TERMINAL SEQUENCE OF CALF FETUIN.

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Received December 10,1980

SUMMARY.

Calf fetuin, one of the 3 major known fetal proteins has been isolated by a two-step purification procedure and characterized by aminoacid composition. The purified glycoprotein, which consisted of a single chain, was submitted to 47 steps of automatic aminoacid sequencing, allowing to determine 44 positions. This section of the molecule was devoid of carbohydrates. Comparison of this sequence with a variety of detectable potentially related protein did not allow to point to any detectable homology.

INTRODUCTION

Calf fetuin was first described by Pedersen in 1944 (1) as a major component of fetal serum. This α-globulin was subsequently identified although in much lesser amounts, in sera from newborn calves (2), with a concentration that gradually decreases during the first weeks of life. Fetuin differs from α feto protein in that it seems until now strictly restricted to the ruminant and probably porcine species (2, 3, 4).

Previous studies allowed determination of molecular weight, aminoacid and carbohydrate composition of calf fetuin (5, 6). Despite some reports regarding a possible biological activity its role remains unclear. Fisher and Lam (7) showed that calf fetuin was able to bind thyroxin and it was demonstrated that the capacity of fetal sera to stimulate adherence and growth was related to the α-globulinic fraction (8, 9, 10). Until now the possible participation of fetuin in the cell growth stimulating properties of fetal calf serum has not been clearly established (11, 6). More recently, calf fetuin was shown to exert a trypsin

0006-291X/81/050030-07$01.00/0

inhibitor activity (12) and this finding was extended to lamb fetuin (Lafont et al., manuscript in preparation).

In order to provide a basis for studying functionally related proteins, the aminoacid sequence of calf fetuin was undertaken. In this paper, we report the identification of the first 44 aminoacid residues of the NH_2-terminal section.

MATERIALS AND METHODS

Preparation of fetuin

Fetuin was obtained from fetal calf serum according to the two-step procedure described by Marti and coworkers for lamb fetuin (13). Briefly, pooled sera were exhaustively dialyzed against 0.01 M sodium acetate, pH 4.6 ; the precipitate (about 20% of the total protein content) was discarded by centrifugation and the supernatant was chromatographed over a CM-52 column. Fetuin was eluted with the same buffer and was further purified by chromatography on Sephadex G 200. The purity was checked by 7.5% polyacrylamide gel electrophoresis (14), isoelectric focusing (15) and crossed-immunoelectrophoresis (16) using antisera prepared as described elsewhere (4).

Oxidation, reduction and alkylation

Disulfide bridges were oxidized with performic acid (17) or reduced with dithiothreitol. In the latter case, the cysteyl residues were alkylated with iodoacetamide (18).

Aminoacid composition

The total aminoacid composition was determined after 24, 48 and 72 hour hydrolysis of native and oxidized protein in 6 N HCl, using a Beckman 121 M analyser ; values obtained for serine and threonine were corrected for acid degradation by extrapolation to zero time ; the aminoacid composition and the molecular weight of the polypeptide chain were calculated according to the method of Delaage (19) using a computer (Hewlett Packard 9825 A).

TABLE I

Aminoacid	Calf fetuin mol/mol of protein	Calf fetuin (from ref. 6)	Lamb fetuin (from ref. 14)
Asx	33.4	33	32
Thr	20.0(1)	25	20
Ser	25.1(1)	26	22
Glx	40.0	34	38
Pro	34.2	34	35
Gly	22.7	24	25
Ala	37.4	33	40
Val	40.9	40	38
Met	0	0	0
Ile	13.0	15	14
Leu	27.1	27	28
Tyr	8.4	7	8
Phe	11.6	11	12
His	8.5	10	11
Lys	14.1	16	16
Arg	11.5	12	11
Half-cystine	11.6(2)	12	12
Trp(5)	2	2	2
Number of residues	362(3)	361	364
Molecular weight	38,925(4)	38,725	39,025

(1) Values obtained by extrapolation to zero time

(2) Analysis of the oxydized derivative : cysteic acid

(3) Sum of the closest integers

(4) As calculated by the method of Delaage (19).

(5) Values taken from ref. 14.

Sequence determination

Direct sequence analysis was obtained with the Beckman 890 C automated sequencer, using a 0.33 M quadrol. PTH derivatives were identified by high performance liquid chromatography (HPLC) on a microbondapak C 18 column and using a Waters liquid chromatograph apparatus. An exponential gradient of methanol-phosphate (gradient curve n° 10) was applied for 10 minutes according to the following conditions : initial

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 1                                                          10
Ile - Pro - Leu - Asp - Pro - Val - Ala - Gly - Tyr - Lys -

                                                           20
Glu - Pro - Ala - Cys - Asp - Asp - Pro - Asp - Thr - Glu -

                                                           30
Gln - Ala - Ala - Leu - Ala - Ala - Val - Asp - Tyr - Ile -

                                                           40
Asn - Lys - His - Leu - Pro - Arg - Gly - Tyr - Val - Lys -

Thr - Leu -(Asn, Gln)
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Figure 1 : Aminoacid sequence of residues 1 to 44 of calf fetuin.

concentration : 22% Methanol - 78% sodium phosphate 0.01 M, pH 7.4 ; final concentration : 42% Methanol - 58% sodium phosphate. The overall run time was 28 minutes. An aliquot of each PTH derivative was hydrolyzed with HI (20) and the corresponding aminoacid residue was identified on the aminoacid analyzer.

RESULTS

The purity of the protein preparation was tested by crossed-immuno-electrophoresis, using a rabbit antiserum anti whole fetal calf serum. Purified calf fetuin migrated as one single band in SDS-polyacrylamide gel electrophoresis. A series of discrete bands near pH 4.0 on isoelectric focusing characteristic of the internal microheterogeneity of fetuin were observed, in agreement with previous reports (21). The mean aminoacid composition of calf fetuin is given on Table I, together with the aminoacid composition determined by Spiro (5) and with that of lamb fetuin (13).

One hundred nanomoles of reduced-carboxymethylated calf fetuin were submitted to automated Edman degradation. Forty-seven steps of degradation were performed, from which 44 residues were identified, accounting for about 12% of the total aminoacid content of the protein. The mean repetitive yeld was 95%. NH_2-terminal sequence of calf fetuin is reported on Figure 1. No evidence was obtained concerning the presence of glycan residues in this section of the molecule.

DISCUSSION

Computation of aminoacid composition of calf fetuin, as obtained by the two-step purification procedure described for lamb fetuin (13) gave a total number of 362 residues accounting for a molecular weight of about 39,000 daltons for the peptidic part of the molecule. This amino-acid composition was in good agreement with that determined by Spiro. A significant relationship was also observed between aminoacid compositions of calf and lamb fetuin.

The amino-terminal sequence of calf fetuin was compared with amino-acid sequences of known proteins including heavy and light chains of immunoglobulin, β2-microglobulin, bovine serum albumin, α and β chains of human haptoglobin, α_1-acid glycoprotein, bovine myoglobin, bovine α and β(adult and fetal) chains of hemoglobin (reviewed in 22) and cyanogen bromide fragments I and II of human α foeto protein (23). We did not succeed in finding any structural homology between calf fetuin and these proteins. An anti-tryptic activity was described for calf (12) and lamb fetuin. Nevertheless, amino-terminal sequence of calf fetuin did not exhibit any homology with the known basic protease inhibitors, including ovomucoid, bovine pancreatic and bovine colostrum trypsin inhibitor. This does not exclude structural similarities between these proteins and other portions of the fetuin polypeptide chain, especially those responsible for the inhibitory function.

Acknowledgement. This work was supported by INSERM and CNRS and by a grant n° 77-7-0658 from DGRST.

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