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STRUCTURAL IDENTITY OF IMMUNOGLOBULIN BINDING FACTOR AND PROSTATIC SECRETORY PROTEIN OF HUMAN SEMINAL PLASMA

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The amino acid sequence of the N-terminus of the immunoglobulin binding factor of human seminal plasma was determined. The initial 30 amino acids showed complete identity with that of prostatic secretory protein, β-microseminoprotein and β-inhibin. In conclusion, these proteins are probably a single entity.

Human seminal plasma contains Ig and Ig Fc binding factors (1-3). One of these factors designated as IgBF with an estimated Mr of 16 kD was purified and characterized (4). In the present study the amino acid sequence of the N terminus and the C terminal amino acid of IgBF were determined. Data are presented showing that the amino acid sequence of the N terminus and the C terminal amino acid of IgBF are identical to that of PSP (5).

EXPERIMENTAL METHODS

Materials

Semen samples were obtained from healthy men. Pooled semen was separated into sperm and plasma by centrifugation. The seminal plasma was collected, lyophilized and stored at -70°C.

Purification of IgBF

Details of the purification steps are described in another report (4). In brief, IgBF was purified from seminal plasma to homogeneity by ammonium sulfate precipitation, preparative isoelectrofocusing and gel filtration chromatography and preparative SDS-PAGE.

Abbreviations: IgBF, immunoglobulin binding factor; PSP, prostatic secretory protein; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; Ig, immunoglobulin.

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Preparation of sample for amino acid sequencing

Purified IgBF was analyzed by SDS-PAGE and transblot onto a PVDF immobilon sheet. IgBF was stained with Ponceau S. The stained protein was cut out and submitted for sequencing.

Sequencing of IgBF

The amino acid sequence of the NH_2 terminus of the 16-kD protein was determined by The Protein Sequencing Facility of the Rockefeller University under the directorship of Dr. Sheenah Mische, using the Applied Biosystems 470A Protein Sequencer equipped with online AB1120A PTH Analyzer and AB1900 Data Analyzers. The carboxyl terminus was established by performing a time course (0-30 min) digests with carboxypeptidase Y (Boerhinger Mannheim) (enzyme/substrate 1:20) of the 16-kD protein. Each time point digestion was terminated with the addition of 1 µl 1% DFP/ethanol (v/v), taken to dryness in a Savant Speed vacuum concentrator and derivatized for PTC amino acid analysis. Appropriate controls for protein and enzyme were performed and analysis performed using a Waters Picotag System and HPLC equipped with WISP and Model 490 Multiwave length detector with 840 data station.

Carboxymethylation

Purified IgBF was reduced and carboxymethylated according to the method of Crestfield et al. (6) Sample of IgBF was incubated in a medium containing 0.05 M Tris-HCl, pH 8.4, 8 M urea, 0.5 mM EDTA, 25.6 mM d-mercaptoethanol and 22.5 µM iodoacetic acid. The carboxymethylated IgBF was analyzed for cysteine residue.

RESULTS

The amino acid sequence of the N-terminal segment of IgBF, PSP (5), the inhibin-like peptide (7-9), and β-microseminoprotein (10) are depicted in Fig. 1. Note that the initial 30 amino acids of the N-terminal segments are ident-

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1                   5                   10                  15
S   C   Y   F   I   P   N   E   G   V   P   G   D   S   T
SER CYS TYR PHE ILE PRO ASN GLU GLY VAL PRO GLY ASP SER THR

16                  20                  25                  30
R   K   C   M   D   L   K   G   N   K   H   P   I   N   (S)
ARG LYS CYS MET ASP LEU LYS GLY ASN LYS HIS PRO ILE ASN SER

----------------------------------I   I
                                  ILE ILE-COOH

     N-terminal                                   C-Terminal

      1        10        20        30              94
A     SCYFIPNEGVPGDSTRKCMDLKGNKHPINSE---------------IICOOH

B     SCYFIPNEGVPGDSTRKCMDLKGNKHPINSE---------------IICOOH

C     SCYFIPNEGVPGDSTRKCMDLKGNKHPINSE----------------ICOOH

D     SCYFIPNEGVPGDSTRKCMDLKGNKHPINSE---------------GICOOH

E     SCYFIPNEGVPGDSTRKCMDLKGNKHPINSE---------------GICOOH
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Figure 1. Amino acid sequence of immunoglobulin binding factor and related proteins isolated from seminal plasma.
A, IgBF; B, prostatic secretory protein (5); C, β-microseminoprotein (10); D, β-inhibin (8); E, Inhibin-like peptide (9).

ical. In addition, the last two amino acids of the carboxyl terminus of IgBF and PSP are isoleucine; whereas that of the other two proteins were reported to be glycine and isoleucine. The present findings suggest that IgBF, PSP, β-inhibin, and β-microseminoprotein are probably one and the same protein.

DISCUSSION

A function of PSP was attributed to that of inhibin-like activity (7). However, this claim was refuted by Kohan et al. (11). We proposed that IgBF/PSP may influence immunological processes because it binds Ig of various species, interacts with anti-Leu-11b antibodies raised against an NK cell antigen (CD16) and with polyclonal anti-FcγRIII antibodies (3,4). These findings suggest that IgBF may regulate lymphocyte function, transport Ig, or modulate NK cell activity (12-14). Our recent study suggests that IgBF suppresses lectin-stimulated lymphoblastogenesis (unpublished data) and may be one of the immunosuppressive factors purported to be present in seminal plasma (15-19).

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