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Detection of Human Spermatozoal Peptides after Conjugation to ^{125}I-labelled Human Serum Albumin

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Abstract

Human spermatozoal peptides, liberated during autolysis of the cells, were fractionated by gel-filtration chromatography and thin-layer chromatography. After conjugation to ^{125}I-labelled human serum albumin, all fractions were assayed with rabbit antihuman spermatozoa antiserum. The reactions indicated the presence of a number of antigenic peptides in the spermatozoal autolysate and one of the fractions revealed a significantly higher antibody binding capacity than all other fractions assayed ($P < 0{\cdot}05$). Release of immunogenic peptides during autolysis of the spermatozoal membrane could be important in the aetiology of spermatozoal iso- and autoantibodies.

Introduction

Earlier investigations have shown that the human spermatozoal membrane carries low molecular weight compounds, which can be released during autolysis of the cells (Mettler and Skrabei 1980). These compounds may arise through digestion of spermatozoal antigens by their own proteolytic enzymes (Schwartz and Koehler 1979). A compound of molecular weight 1800 was obtained earlier by extraction of the spermatozoa with Hyamine 2389 and Triton X-100, followed by chromatographical purification (Mettler and Skrabei 1979). The fraction inhibited the sperm-immobilizing action of sperm antisera and after conjugation to a high molecular weight carrier exhibited an immunoprecipitation reaction with sperm-immobilizing human sera. The low sensitivity of the immunoprecipitation reaction and the microsperm immobilization test (Mettler 1977) necessitated a more sensitive test.

The purpose of the present work is to describe a solid-phase radioimmunoassay for the detection of antigenic peptides.

Materials and Methods

Autolysis of human spermatozoa was carried out as described by Mettler and Skrabei (1980).

Chromatography on Biogel P-4

Chromatography of the spermatozoal extract was performed on a Biogel P-4 (Bio-Rad, Munich, Germany) column (1·7 by 83 cm) which was equilibrated and eluted into 6-ml fractions with 1% (w/v) acetic acid. Approximately 600 mg of the freeze-dried material was applied to the column and 6-ml fractions were collected at a flow rate of 3 ml/h. The column was calibrated with bradykinin (mol. wt 1040), bacitracin (mol. wt 1450) and gramicidin (mol. wt 2000).

Thin-layer Chromatography

Every third fraction was lyophilized and resuspended in 50 μl distilled water. Consecutively the solutions were applied on a line 1 cm from the lower edge of a cellulose plate (Cel 400, 10 by 10 cm; Machery-Nagel, Düren, Germany) and ascending chromatography was performed in the solvent system acetic acid–pyridine–butanol–distilled water (30 : 100 : 150 : 120 v/v). After the thin-layer chromatography (t.l.c.) a small strip at the centre of the chromatogram was stained with ninhydrin.

In the area where bands appeared the cellulose layer was scraped off the plate and the peptides were eluted with 1 ml of a 1% (w/v) acetic acid solution for 1 h. The mixture was centrifuged for 5 min at 300 *g*, the supernatant lyophilized and redissolved in 50 μl of distilled water.

Labelling of Human Serum Albumin (HSA) with ^{125}I

Labelling was done according to the method described by Schulze (1976).

Conjugation of the Peptides to Labelled HSA

The peptides were conjugated to the labelled HSA by the following procedure: 20 μl of the ^{125}I-labelled HSA solution (20 μg), was placed in a plastic tube with 50 μl of 1-ethyl-3-(3-dimethyl-aminopropylcarbodiimide)hydrochloride (0·75 μg). The reaction was allowed to proceed for 12 h at room temperature, pH 4·5, after which 0·5 ml of a dextran-coated charcoal suspension in distilled water (40 μg/ml) was added and the mixture incubated for 1 h. The suspension was centrifuged and the supernatant separated.

Antiserum Titres

Antiserum titres were determined by the microsperm agglutination (MSA) and by the microsperm immobilization (MSI) tests (Hustedt and Hjort 1974; Friberg 1974). In absorption experiments 20 μl of the antiserum was incubated with either an equal volume of peptide solution (1 mg/ml) or with *c.* 10^7 spermatozoa after autolysis. Following incubation, 1 h at 37°C and 24 h at 4°C, the mixture was centrifuged at 5000 *g* and the titres estimated by the above methods.

Reaction of the ^{125}I-labelled Albumin Peptide Conjugates with Fixed Rabbit Antisperm Antiserum

Rabbit anti-human spermatozoa antiserum (Behringwerke AG, Frankfurt, Germany) with a sperm-immobolizing titre of 1:32 and a sperm-agglutining titre of 1:64, was diluted with 0·1 M $NaHCO_3$ buffer, pH 9·6, and 0·5 ml quantities were pipetted into plastic tubes. The tubes were incubated for 2 h at room temperature and then each tube was rinsed twice with 0·5 ml of an ice-cold 0·14 M NaCl solution, followed by a 1 h incubation with 0·5 ml of a solution containing 0·5% (w/v) HSA in 0·01 M phosphate buffer, pH 7·4. The plastic tubes were filled with 0·5 ml of ^{125}I-labelled albumin–peptide solutions, incubated with 0·5 ml of 0·1 M NaCl and subsequently counted in a gamma counter. All assays were carried out in replicates of 10. Labelled unconjugated HSA, or rabbit antihuman spermatozoa antiserum substituted by normal rabbit serum, served as controls. Fractions 27.4 and 39.5 were also assayed in the presence of an excess amount of protein A. The tubes were preincubated with 50 μg of protein A just prior to the addition of the ^{125}I-labelled HSA conjugated peptides. The assays were then carried out the same way as the other fractions.

Results

The elution profile of the spermatozoal autolysate resulted in three peaks. The largest of the peaks chromatographed in a position of molecular weight less than 1040 (Fig. 1). Fractions 24–75 were further separated by t.l.c. into 65 different peptides (Fig. 2). After conjugation to labelled HSA by the carbodiimide method all 65 peptides were tested in the described immunoassay.

An average tracer absorption of 29·7 (±2·5 s.d.) resulted from the various fractions. Fraction 39.5 showed the highest number of counts per minute, 37·4 (±9·7 s.d.) (Table 1). When compared with all the other fractions this fraction

proved to be significantly higher ($P < 0{\cdot}05$, Student's t-test). The second highest tracer absorption was shown by fraction 27.4 with counts per minute of 34·4 (±11·2 s.d.).

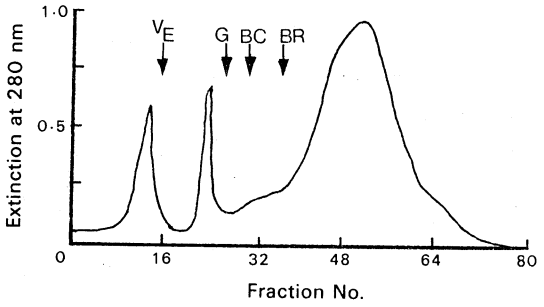

Fig. 1. Elution profile of the spermatozoal autolysate on a Biogel-P4 column. Experimental details as described in the Materials and Methods section. *G*, gramicidin; *BC*, bacitracin; *BR*, bradykinin; V_E, exclusion volume.

However, comparing this fraction with the average tracer absorption no significant difference was found ($P < 0{\cdot}1$). Substitution of the rabbit anti-human spermatozoal antiserum by normal rabbit serum, or using unconjugated labelled HSA instead of the conjugated compounds, resulted in a fraction with counts per minute of 25 (±2·7 s.d.).

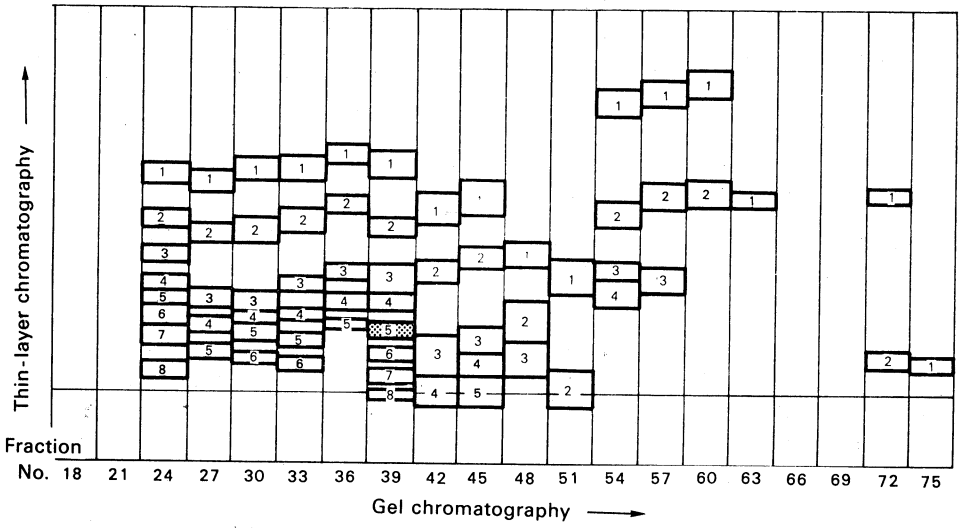

Fig. 2. Schematic representation of the separation of low molecular weight compounds from human spermatozoa by chromatography on Biogel P4 and ascending thin-layer chromatography on Cel 400. Experimental details as described in the Material and Methods section. Horizontal line indicates sample application. Numbers 1–8 are relative R_F values. Numbers 18–75 are fraction numbers after gel-filtration chromatography. Each band was eluted and tested by radioimmunoassay (see Table 1). The stippled area represents reaction with rabbit anti-spermatozoa serum.

Tracer absorptions for the fractions 27.4 and 34.4 in the presence of protein A were not significantly different from those without protein A. In the absorption experiments, the titre of the rabbit antihuman sperm antiserum was significantly

reduced (greater than two dilution steps) with both, the spermatozoal autolysate and with the cells remaining after autolysis. Absorption with the autolysate, however, was more pronounced than with the cells, 1:4 *v.* 1:16 in MSA and 0 *v.* 1:8 in MSI.

Table 1. Reactions of spermatozoal peptides conjugated to ^{125}I-labelled HSA with rabbit antihuman spermatozoal serum

*$P < 0.05$

Fraction No.	Immunoassay (counts/min)[A]	Fraction No.	Immunoassay (counts/min)[A]	Fraction No.	Immunoassay (counts/min)[A]
24.1	27·5 ± 3·5	33.4	27·7 ± 3·6	45.3	31·1 ± 2·9
24.2	31·3 ± 5·5	33.5	25·6 ± 3·2	45.4	31·9 ± 5·8
24.3	26·8 ± 2·5	33.6	27·9 ± 3·5	45.5	29·6 ± 8·3
24.4	29·8 ± 5·7	36.1	31·4 ± 9·6	48.1	31·9 ± 4·1
24.5	24·6 ± 2·3	36.2	25·0 ± 3·6	48.2	30·0 ± 5·1
24.6	32·0 ± 4·2	36.3	31·1 ± 7·7	48.3	30·6 ± 3·1
24.7	25·4 ± 1·9	36.4	33·1 ± 4·7	51.1	28·3 ± 3·6
24.8	29·8 ± 3·0	36.5	29·1 ± 3·3	51.2	27·1 ± 3·5
27.1	33·5 ± 4·5	39.1	31·7 ± 5·5	54.1	28·8 ± 4·9
27.2	32·2 ± 7·6	39.2	30·8 ± 8·1	54.2	31·5 ± 6·2
27.3	30·2 ± 1·5	39.3	29·1 ± 7·5	54.3	27·9 ± 2·4
27.4	34·4 ± 11·2	39.4	29·3 ± 3·4	54.4	28·9 ± 5·4
27.5	29·0 ± 8·8	39.5	37·4 ± 9·7*	57.1	31·0 ± 4·3
30.1	30·4 ± 4·8	39.6	29·8 ± 5·5	57.2	28·8 ± 3·7
30.2	31·0 ± 4·3	39.7	30·1 ± 5·4	57.3	28·2 ± 1·8
30.3	30·5 ± 4·0	39.8	28·1 ± 3·4	60.1	28·5 ± 5·2
30.4	28·3 ± 3·9	42.1	27·6 ± 5·5	60.2	25·9 ± 4·9
30.5	25·6 ± 3·4	42.2	28·3 ± 6·2	63.1	26·2 ± 1·4
30.6	27·9 ± 3·7	42.3	29·5 ± 6·5	72.1	27·8 ± 2·5
33.1	27·8 ± 2·3	42.4	30·6 ± 4·5	72.2	31·0 ± 7·5
33.2	27·8 ± 3·2	45.1	32·4 ± 9·1	75.1	27·9 ± 3·9
33.3	28·1 ± 5·0	45.2	28·6 ± 4·4		

[A] Mean of 10 assays ± s.d.

Discussion

In earlier publications (Mettler 1977; Mettler and Skrabei 1979) human sperm-immobilizing and sperm-agglutinating sera were used for the detection of solubilized spermatozoal antigen. In the present paper the reaction of ^{125}I-labelled HSA conjugates with spermatozoal peptides is described for the first time. The active fraction is revealed in the same chromatographical fraction as the one described earlier.

The labelled HSA conjugate of fraction 39.5 shows a significantly higher binding capacity to the rabbit antihuman spermatozoa antiserum, although the presence of a number of various conjugates with elevated antibody binding is also evident (Table 1).

The antigenic character of the peptides is also demonstrated by the fact that protein A did not inhibit antibody binding. This suggests that the conjugates are actually bound to the 'Fab' region of the antibody molecules. The fact that the antispermatozoal activity of the rabbit antihuman sperm antiserum could be signficantly reduced by absorption with the spermatozoal autolysate, as well as with the whole spermatozoa after autolysis, suggest that not all the peptides are liberated during the autolysis. Further analysis is needed to evaluate this fraction for its exact chemical composition, with a later goal of eventual synthesis.

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