57. *Human Sweat-Specific Antigen*

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Most of protein components in human sweat were immunologically remained obscure. Our present report deals with the demonstration of glycoprotein antigen specific for human sweat.

Materials and methods. Undergarments worn by healthy adults for one day in summer were immersed in distilled water, and spun by Kokusan Centrifuge Type H 110A. The resultant extracts were concentrated by freeze-drying and after transferring into Visking tube, dialyzed overnight at 4°C against distilled water. To one volume of the content, four volumes of a chloroform-methanol mixture (1:1, v/v) were added. The mixture was stirred at room temperature for 10 min and centrifuged at 1,500 rpm for 15 min. The aqueous layer occurred was carefully collected. Twenty volumes of acetone were added to one volume of the aqueous phase. Resulting precipitates were collected by centrifugation at 3,000 rpm for 15 min and dialyzed overnight against distilled water. The non-dialyzable portion was freeze-dried.

Five mg of the dried preparation were dissolved in 0.5 ml of physiological saline and then mixed with an equal volume of Freund's complete adjuvant. The mixture was injected subcutaneously into a Japanese albino rabbit, five times biweekly. After two weeks from the final injection, the animal was bled and the serum was separated. Serological specificities of antibodies produced against human sweat were examined by precipitation ring test.

Five hundred mg of the crude sweat material were applied on a column (1.5×20 cm) packed with DEAE Sepharose CL 6B (Pharmacia Fine Chemicals) which was equilibrated with 0.02 M Tris-HCl buffer, pH 7.2. The protein materials from sweat were eluted stepwise by the same buffer with increasing NaCl concentrations, 0.05 M, 0.1 M, 0.3 M, 0.5 M and 1.0 M NaCl, respectively. The materials eluted from the DEAE Sepharose column were further purified by gel filtration chromatography, using a column packed with Bio Gel P-60 (Bio Rad Co., 1.5×84 cm). Molecular weight calibration was carried out using protein standards of bovine albumin (67,000), ovalbumin (45,000), chymotrypsinogen A (25,700) and cytochrome *c* (13,000).

Isoelectric focusing was performed on a polyacrylamide gel plate using carrier ampholyte of pH range 3–10 (Pharmalyte). The procedures of electrophoresis and gel staining were similar to those in the previous report.¹⁾ Gradient gel electrophoresis was performed on a Pharmacia's PAA 4/30 plate.

Results. The immune serum reacted not only with human sweat, but also with serum, saliva, seminal fluid, vaginal fluid and urine. For preparing a sweat-specific antiserum, human serum was added to the immune serum until the mixture failed to produce additional precipitates against human serum. The immune serum absorbed with human serum precipitated a crude sweat preparation at a minimal concentration of 0.0001% (w/v) but did not react with any other body fluid tested and with any type of purified blood group substances. The antigen specifically reacting with the absorbed antiserum was named as sweat-specific antigen (SSA) and some properties of this antigen were elucidated as follows.

Fig. 1. Gel filtration pattern of SSA fraction from DEAE Sepharose column chromatography.

1) *Thermostability.* A solution of crude sweat preparation in saline was heated at 100°C in water bath. Even after 60 min, no remarkable decrease of the antigenicity was observed.

2) *Purification of SSA.* Sweat-specific antigen was eluted from the DEAE Sepharose column with 0.02 M Tris-HCl buffer, pH 7.2, containing 0.1 M and 0.3 M NaCl. Both antigenic fractions were pooled and further purified by gel filtration as shown in Fig. 1.

Sweat-specific antigen appeared as a single peak at elution volume of 56 ml. The molecular weight of SSA was estimated to be about 30,000 by the calibrated column.

3) *Electrophoresis*. When the SSA fraction purified on the Bio Gel P-60 column was analyzed by isoelectric focusing, a broad band located between soybean trypsin inhibitor (pI 4.55) and amyloglucosidase (pI 3.50) was visualized on the polyacrylamide gel plate by Coomassie Blue staining (Fig. 2).

Fig. 2. Isoelectric focusing pattern of SSA. Lane 1: Calibration kit proteins. Lane 2: SSA. Coomassie Blue staining.

On the gradient gel electrophoretogram, SSA migrated fast to the anode and gave no band similar to those derived from human serum.

4) *Specific staining for SSA*. SSA was positively stained with PAS method, but negatively with 1% (w/v) toluidine blue solution for acid mucopolysaccharide detection.

Discussion. The anti-human sweat-specific precipitin was demonstrated in the rabbit antiserum against crude sweat preparation. The antigen (SSA) was thermostable and appeared to be a glycoprotein with approximate molecular weight of 30,000 and with pI 4.

Jirka and Masopust²⁾ obtained a rabbit antiserum by injecting a glycoprotein fraction of human thermal sweat. On Ouchterlony precipitation test, they observed two precipitin lines against human sweat which did not correspond to the precipitin lines found against human serum.

Herrmann and Habbig³⁾ reported that there were three protein fractions in human eccrine sweat migrating like α -, β - and γ -globu-

lins on cellulose acetate membrane electrophoresis and they found eight precipitin lines appeared on the immunoelectrophoresis using a rabbit antiserum against human sweat proteins. They have mentioned that one of the eight lines could be epidermal origin and another one seemed to be eluted from the sebum film.

Conclusion. We succeeded in demonstrating a precipitin specifically reactive with human sweat in a rabbit antiserum. An antigen corresponding to it was seemed to be a glycoprotein with approximate molecular weight of 30,000 and with pI 4.

References

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