TECHNICAL NOTE

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A New Individualization Marker of Sweat: Deoxyribonuclease I (DNase I) Polymorphism*

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ABSTRACT: We have confirmed for the first time, both biochemically and immunologically, the existence of deoxyribonuclease I (DNase I) in human liquid sweat. Isoelectric focusing of sweat samples on polyacrylamide gels (pH 3.5 to 5), followed by dried agarose film overlay detection, was used to determine the phenotypes of sweat DNase I. Because this detection method not only had high sensitivity, but also high band resolution, it was possible to determine DNase I types from sweat samples of 50 to 100 μL. Pretreatment of sweat samples with sialidase was essential for typing to enhance markedly the sensitivity accompanied by simplification of the isozyme pattern. The DNase I types in all sweat samples were consistently related to the types found in corresponding blood, urine, and semen samples. DNase I typing could, therefore, provide a novel discriminant characteristic in the forensic examination of sweat.

KEYWORDS: forensic science, phenotyping, body fluids, deoxyribonuclease I (DNase I), dried agarose film overlay (DAFO), isoelectric focusing, single radial enzyme diffusion, sweat

Genetic polymorphism of human deoxyribonuclease I (DNase I; EC 3.1.21.1) has been detected in body fluids and tissues by means of isoelectric focusing. The DNase I phenotypes are known to be controlled by four codominant alleles, *DNASE1*1*, **2*, **3*, and **4*, whose gene frequencies have been calculated in a Japanese population to be 0.552, 0.436, 0.010, and 0.002, respectively (1,2). The gene symbol in the Genome Data Base (GDB) is *DNL1*. In view of the favorable distribution of its gene frequencies, high stability of the enzyme protein, and high enzyme activity in human biological fluids, DNase I has been used as one of the most valuable genetic markers for forensic, genetic, and clinical investigations (3–6). Recently, its genomic structure (7), chromosomal assignment (8), and the molecular basis for its genetic polymorphism (9,10) have been elucidated in our laboratory. As DNase I is distributed widely in biological fluids such as breast milk, serum, saliva, semen, and urine (5,6,11,12), its polymorphism is useful as an individualization marker of such fluids. Only the ABO system has been used so far as a genetic marker for individualization of sweat. Therefore, there is a need for a new and more effective system of discrimination in sweat/sweat stain samples in addition to the ABO system.

In this technical note, we describe the results of a trial to determine the DNase I phenotype from liquid sweat samples and the correlation of DNase I types among sweat, blood, urine, and semen samples from the same individuals.

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Materials and Methods

Biological Samples

A set of sweat, blood, semen, and urine samples were collected from individual healthy Japanese donors. Liquid sweat samples were collected from the forehead during light physical work, dialyzed against 0.1% (w/v) glycine, and lyophilized. A 2% (w/v) solution of the lyophilized material, corresponding to 10- to 15-fold concentrated sweat, was used for DNase I phenotyping. Venous blood was taken into tubes containing heparin, and the serum was separated. Urine samples were concentrated, dialyzed and finally lyophilized, as described previously (1,13). A 0.1% (w/v) solution of the lyophilized material corresponding to tenfold concentrated urine was used for DNase I typing. Semen samples were collected as described previously (12) and diluted 1:4 with distilled water. These solutions were treated with an equal volume of 10 units/mL *Clostridium perfringens* sialidase (Sigma, St. Louis, MO) in 50m*M* sodium acetate buffer, pH 5.0, before electrophoresis. The digest of 5–10 μL was used for isoelectric focusing on a thin layer of polyacrylamide gel (IEF-PAGE).

DNase I Phenotyping

Isoelectric focusing was performed according to the previous method (13) with a slight modification. Gels measuring 0.5 (thickness) by 90 (width) by 120 (length) mm were prepared using the

following materials: 1.4 mL of acrylamide-*N,N'*,-methylenebisacrylamide (19.4%, w/v; 0.6%, w/v), 1 mL of distilled water, 2.3 mL of sucrose-glycerol (20%, w/v; 10%, v/v), 280 μL of Ampholine 3.5–5 (Pharmacia Biotech, Uppsala, Sweden), 5 μL of *N,N,N',N'*,-tetramethylethylenediamine and 40 μL of 1.2% (w/v) ammonium persulfate. Wicks were formed from strips of filter paper and soaked in the electrode solution; 1.0*M* phosphoric acid (H_3PO_4) at the anode and 2% (v/v) Ampholine 5–7 (Pharmacia Biotech) at the cathode, respectively. A sample was applied to the gel with a Whatman 3MM filter paper at a distance of 20 mm from the cathode wick. A Multiphor apparatus (Pharmacia Biotech) was used to run the gel at V_{max} 1000 V, I_{max} 10 mA, P_{max} 3 W for 4 h under cooling at 12°C.

Visualization of DNase I was achieved using the dried agarose film overlay (DAFO) method (3,13). For this, the reaction mixture consisted of 0.05 mg of ethidium bromide and 0.05 mg of salmon testis DNA (type III, Sigma) per 1 mL of 100m*M* sodium cacodylate buffer, pH 6.5, which contained 0.2m*M* calcium chloride ($CaCl_2$) and 2.0m*M* magnesium chloride ($MgCl_2$). To the reaction mixture, an equal volume of 2% (w/v) molten agarose in distilled water at 50°C was added, mixed, and poured immediately onto a horizontal Agafix sheet (Wako Pure Chemical Industries, Ltd., Osaka, Japan). After solidification at room temperature, the agarose gel was dried completely in an incubator at about 50°C to produce a dried agarose film. After the IEF-PAGE run, the dried agarose film was placed carefully on the top of the focused gel in full contact. The IEF-PAGE gel was incubated in contact with the agarose film at 37°C and the progress of DNase I action was observed under UV transillumination (312 nm). Ethidium bromide produces fluorescence with intact, but not with degraded DNA digested by DNase I. After incubation for optimal development, the film was removed from the gel and then observed or recorded photographically. DNase I types in serum, semen, and urine samples were analyzed by the previous methods (2,3,13).

Activity Assay and Characterization of Sweat DNase I

Sweat DNase I activity was determined by the single radial enzyme diffusion (SRED) method reported by Nadano et al. (11). Effects of G-actin, anti-human DNase I antibody and divalent cations on sweat DNase I activity were examined as described previously (14,15).

Results and Discussion

DNase I types are valuable in terms of discrimination potential (1,2) and high stability of the enzyme protein. High activity levels of DNase I were observed in 20 sweat samples by the SRED method. The enzyme activity was completely inhibited by 1m*M* ethylenediaminetetraacetate (EDTA) and also by 1m*M* ethylene glycol bis(aminoethyl ether) tetraacetic acid (EGTA) even in the presence of 10m*M* $MgCl_2$. G-actin, known to be a potent inhibitor of bovine-pancreatic and human DNase I (14,15), completely inhibited the activity of sweat DNase I. Furthermore, the activity was completely abolished by anti-human DNase I antibody, but not by anti-human DNase II antibody. It was clarified that these catalytic and immunological properties of the sweat DNase I were very similar to those of the urinary and seminal enzymes (6,14). Human sweat contains α-amylase and alkaline phosphatase, as well as renin-like activity (16). This is the first study to confirm the presence of DNase I activity in human sweat.

The mean activity of sweat DNase I was determined to be 29.1 ± 12.0 (mean ± SD) × 10^{-3} units per mg protein of sweat (range, 15 to 47 × 10^{-3}), which was estimated simultaneously in terms of the sweat volume to be 0.98 ± 0.53 units/L.

Although the activity level of sweat DNase I per unit volume was lower than those for other biological fluids such as urine, semen, and saliva, the mean activity level per mg of sweat protein was almost equivalent to those of fluids other than urine (Table 1). Therefore, it was concluded that the enzyme activity level in sweat was sufficiently high for phenotyping by our method using a combination of IEF-PAGE and the zymogram method because we succeeded in typing DNase I from serum samples with a similar level of enzyme activity.

The DNase I types of 20 Japanese donors were determined using both urine and sweat samples, and the results agreed with the donors' types in each case. The IEF-PAGE pattern of sweat DNase I was similar to those of serum, urinary, and seminal DNase I in each type, as shown in Fig. 1. This finding was supported by the fact that a mixture of serum and sweat of Type 1 (or Type 2) gave a single main isozyme band indistinguishable from that of Type 1 (or Type 2) serum on the gel (data not shown). Pretreatment of sweat samples with sialidase was essential for precise and sensitive typing of sweat DNase I in the same manner as DNase I typing in other biological fluids (1–3,5). DNase I types were examined in several sets of sweat, semen, serum, and urine from the same donors. The type of each donor was determined from the sweat samples, and the results agreed with the donors' types in each case; no example of noncorrelation of DNase I typing among

TABLE 1—*DNase I activity in human biological fluids.*

Biological Fluid	Activity*		Reference
	units/mL liquid	units/mg protein	
Sweat	(0.98 ± 0.53) × 10^{-3}	29.1 ± 12.0	This study
Saliva	0.56 ± 0.56	18.7 ± 12.6	(5)
Semen	0.35 ± 0.17	32.1 ± 17.1	(6)
Serum	(4.40 ± 1.80) × 10^{-3}	65.0 ± 27.0	(11)
Urine	0.60 ± 0.22	6000 ± 2200	(11)

*Activities are given as mean value ± SD.

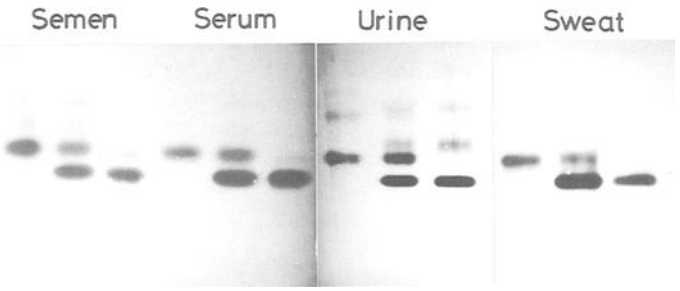

FIG. 1—*Isoelectric focusing (pH 3.5–5) patterns of three common DNase I phenotypes from desialylated semen (lanes 1, 2 and 3 from left to right), serum (lanes 4, 5 and 6), urine (lanes 7, 8 and 9) and sweat (lanes 10, 11 and 12) samples detected by activity staining using the DAFO method (see Materials and Methods). Anode is at the top. Paired samples obtained from the same donors: lanes 1, 4, 7 and 10, type 1; lanes 2, 5, 8 and 11, type 1–2; lanes 3, 6, 9 and 12, type 2.*

corresponding semen, serum, and urine samples was found. When typing the sweat samples, three serum samples of known Types 1, 1–2, and 2 should be simultaneously applied to the IEF-PAGE gel as a control.

We determined the minimum detection limits of liquid sweat for DNase I typing. The volume of liquid sweat required to type the heterozygote (Types 1 and 2) was approximately twice that for the homozygote (Types 1 and 2). It proved possible to determine DNase I types from about 50 to 100 μL of liquid sweat. Longer incubation with the DAFO sheet enabled DNase I to be typed from a much smaller volume of liquid sweat.

Sweat is known to be secreted by two types of sweat gland, the small eccrine glands and the larger apocrine glands. The eccrine sweat glands are far greater in number and found mostly in hairless skin areas, whereas the apocrine ones are found more in hairy regions. Because liquid sweat samples were collected from the forehead of each donor, where eccrine sweat glands are predominantly distributed, it is reasonable to postulate that DNase I might be secreted from eccrine sweat glands. We have already succeeded in specific detection of human sweat using an immunological method (17). Up to now, limited genetic markers, ABO, secretory/nonsecretory (18) and Sd^a (19) systems have been found in human sweat. Among them, only ABO system is commonly used for individualization of sweat. The present findings indicate that the DNase I isozymes in sweat, serum, semen, and urine are comparable, and that typing of DNase I could therefore provide a novel discriminant characteristic in sweat samples. The value of such a test in forensic applications will depend on the availability of typing in sweat stains rather than liquid sweat. Investigation of DNase I typing from sweat stains is currently in progress in our laboratory.

Although DNA polymorphism can now be applied to the individualization of body fluids bearing in mind its high power of discrimination, it may be difficult to perform any DNA typing from sweat samples with no nucleated cells. Therefore, DNase I, which can be detected by traditional, but well approved and well established methods with an unquestioned genetic basis, is very effective and indispensable for criminal investigation of sweat samples.

Conclusion

Use of DNase I polymorphism for individualization of sweat could offer useful information for practice forensic biologists in addition to the use of the ABO system because of the relatively high activity level and favorable gene frequency of sweat DNase I.

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