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DIA3 Phenotyping in Human Tissues, Dental Pulps, and Hair Roots by Isoelectric Focusing

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Summary. The polymorphism of DIA3 was investigated in tissues of various human organs, dental pulps, and hair roots by isoelectric focusing. DIA3 types were demonstrated from tissues of brain, prostate, testis, ovary, and uterus, but not from tissues of spleen, pancreas, heart, liver, muscle, lung, skin, and kidney. Determination was possible from dental pulps stored at room temperature for up to 2 weeks and from fresh hair roots. The results show that the DIA3 typing by isoelectric focusing is useful for medicolegal individualization of brain, reproductive organs, teeth, and hairs.

Key words: Isoelectric focusing, DIA3 polymorphism – DIA3 phenotyping, in organ tissues – DIA3 phenotyping, in dental pulps – DIA3 phenotyping, in hair roots

Zusammenfassung. Mittels Isoelektrofokussierung wurden die DIA3-Typen an verschiedenen menschlichen Organgeweben, Zahnpulpen und Haarwurzeln untersucht. Die DIA3-Typen wurden aus Geweben von Gehirn, Prostata, Hoden, Ovarium und Uterus nachgewiesen, aber aus Geweben von Milz, Pankreas, Herz, Leber, Muskel, Lunge, Haut und Niere nicht. Die DIA3-Bestimmung gelang an bis zu 2 Wochen bei Zimmertemperatur gelagerten Zahnpulpen und an frischen Haarwurzeln. Die Ergebnisse zeigen, daß die DIA3-Typisierung mittels Isoelektrofokussierung zur rechtsmedizinischen Individualisierung von Gehirn, Geschlechtsorganen, Zähnen und Haaren von Nutzen ist.

Schlüsselwörter: Isoelektrofokussierung, DIA3-Polymorphismus – DIA3-Typisierung, an Organgeweben – DIA3-Typisierung, an Zahnpulpen – DIA3-Typisierung, an Haarwurzeln

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Introduction

By means of electrophoresis human diaphorases are separated into multiple isoenzyme components which are controlled by genes at three autosomal loci, DIA1, DIA2, and DIA3 (Hopkinson et al. 1970; Fisher et al. 1977). The DIA1 isoenzymes require NADH and DIA2 NADPH as the electron donor, but the DIA3 isoenzymes accept both NADH and NADPH (Fisher et al. 1977).

Since the discovery of DIA3 polymorphism in human semen (Caldwell et al. 1976), the common phenotypes determined by three codominant alleles, DIA3*1, DIA3*2, and DIA3*3, have been demonstrated also in fetal tissues, placenta, adult brain, and gonads (Fisher et al. 1977; Kühnl et al. 1977; Edwards et al. 1979). We have recently developed an improved isoelectric focusing method for phenotyping DIA3 and have shown that this system has potential as an individualizing marker in the analysis of aged seminal stains (Kido et al. 1987).

This paper describes further application of this technique to the determination of DIA3 types in human tissues, dental pulps, and hair roots for medicolegal purpose.

Materials and Methods

Organ Tissues. The following human tissues were obtained from 11 cadavers (six men and five women) who were medicolegally autopsied within 48 h after death: spleen, pancreas, heart (cardiac muscle), liver, muscle (M. rectus abdominalis), lung, skin (including adipose tissue), kidney, brain, prostate, testis, ovary, and uterus. A small piece of tissue was minced and homogenized in an equal volume of distilled water using an Ultra-Turrax homogenizer (Janke & Kunkel KG, Staufen im Breisgau, FRG). The homogenates were centrifuged at 18,000 rpm for 60 min, and the supernatants were retained for analysis.

Teeth. Teeth were extracted from 53 patients who received treatment at the Dental Clinic of Yamanashi Medical University Hospital. Twenty-nine samples were examined immediately after extraction, 19 samples after storage at room temperature for 1 week, and five samples for 2 weeks. The tooth was crushed with a hammer, and the dental pulp was picked out from the pulp cavity. The pulp tissue weighing 10–20 mg was mashed with a glass rod on a hollowed glass plate in a minimum amount (10–20 µl) of 1% Triton X-100.

Hair Roots. Human scalp hairs were plucked from 25 adult subjects of both sexes. Hair roots apparently bearing sheath cells were macerated on a hollowed glass plate in a minimum amount (about 10 µl) of 1% Triton X-100 and repeatedly compressed with a glass rod in order to lyze the hair sheath cells.

Isoelectric Focusing and Enzyme Staining. The detailed procedure for isoelectric focusing and enzyme staining was described in our previous report (Kido et al. 1987). Both NADH and NADPH (Oriental Yeast, Tokyo, Japan) were used as the electron donor in the staining.

Results and Discussion

Organ Tissues

By the present isoelectric focusing technique and with the use of NADPH as the electron donor DIA3 types were determined in tissue samples of brain, pros-

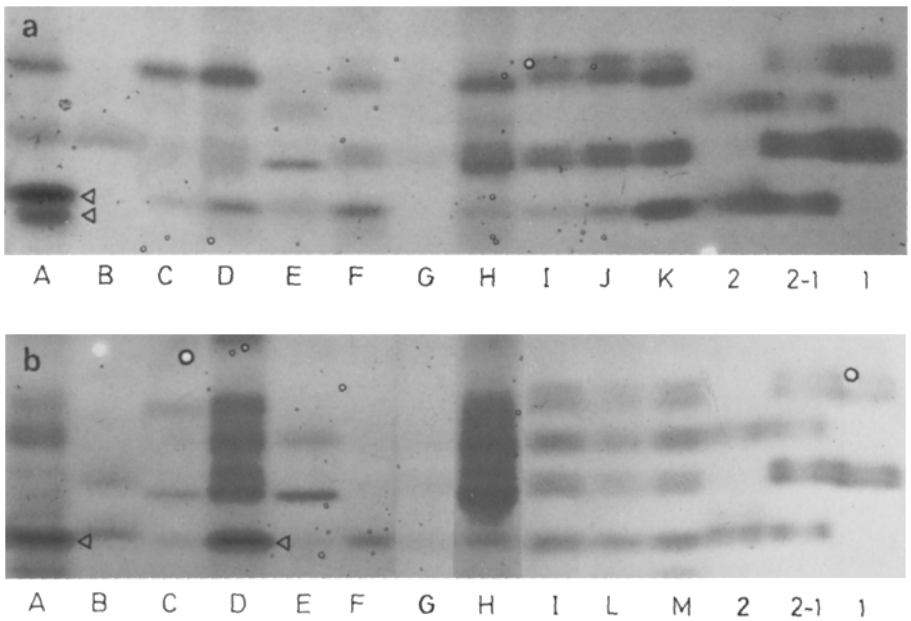

Fig. 1a, b. Isoelectric focusing patterns of DIA3 types in fresh human tissues. NADPH-staining. **a** male, DIA3 1; **b** female, DIA3 2–1. *A* spleen; *B* pancreas; *C* heart; *D* liver; *E* muscle; *F* lung; *G* skin; *H* kidney; *I* brain; *J* prostate; *K* testis; *L* ovary; *M* uterus; *2* control semen sample for DIA3 2; *2–1* control semen sample for DIA3 2–1; *1* control semen sample for DIA3 1. The bands marked ◁ are hemoglobins. The anode is at the *top*

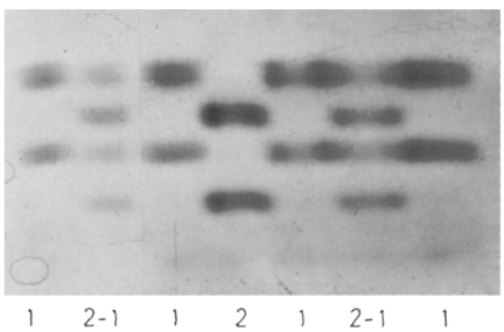

Fig. 2. Isoelectric focusing patterns of DIA3 types in fresh dental pulps. NADPH-staining. The anode is at the *top*

tate, testis, ovary, and uterus (male: five DIA3 1, one DIA3 2–1; female: two DIA3 1, two DIA3 2–1, one DIA3 2). The results agree well with those reported by Kühnl et al. (1977). Spleen, pancreas, heart, liver, muscle, lung, skin, and kidney exhibited various electrophoretic patterns different from the common DIA3 phenotypes, as shown in Fig. 1. The intensity and banding pattern were variable and were not consistent when compared tissue-to-tissue.

According to the evidence presented by Fisher et al. (1977) these components observed in most tissues other than brain and reproductive organs are to be classified into the isoenzymes coded at the DIA3 locus since the patterns still appeared when NADH was used as the electron donor. However, in the

Table 1. Positive results for the determination of DIA3 types in dental pulps

Period of storage	No. tested	Phenotype			
		1	2–1	2	3–1
Fresh	29	20	7	2	0
1 week	19	8	7	3	1
2 weeks	5	3	2	0	0

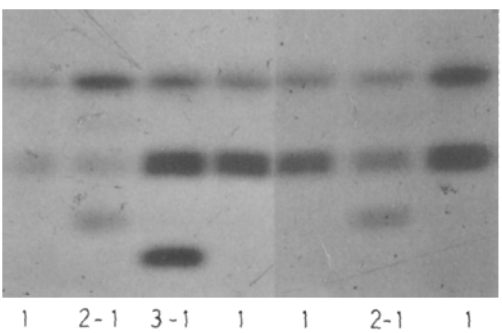

Fig. 3. Isoelectric focusing patterns of DIA3 types in fresh hair roots. NADH-staining. The anode is at the *top*

NADH-staining DIA1 components present in tissues overlapped the DIA3 bands, which made it difficult to interprete the patterns even in brain, prostate, testis, ovary, and uterus. The above NADH/NADPH-dependent tissue isoenzymes seem unlike the NADH-dependent DIA1 and unlike the NADPH-dependent DIA2. Their nature is at present entirely obscure and remains to be elucidated.

In conclusion, the DIA3 typing by isoelectric focusing cannot be utilized for medicolegal individualization of organ tissues except adult brain, prostate, testis, ovary, and uterus.

Dental Pulps

The DIA3 isoenzymes were cleary demonstrated from dental pulps using NADPH (Fig. 2), and reliable phenotyping was possible not only from fresh samples but also from samples stored for up to 2 weeks (Table 1). When stained with the use of NADH, typing was unsuccessful owing to the overlapping of DIA1 components on the DIA3 patterns.

The DIA3 typing by isoelectric focusing and the NADPH-staining would provided a powerful means for the personal identification of teeth provided that brain, prostate, testis, ovary, or uterus is available from the corpse in question.

Hair Roots

The DIA3 patterns in fresh hair roots revealed by the NADPH-staining were distinct (Fig. 3), but so faint that more than five hair samples were needed to ob-

Table 2. Positive results for the determination of DIA3 types in hair roots

Period of storage	No. tested	Phenotype			
		1	2–1	2	3–1
Fresh	25	15	8	0	2
1 week	25	1	1	0	0

tain readable patterns. Phenotyping was also possible in the NADH-staining without interference of DIA1 components. This is probably due to the relatively small amount of blood constituents present in the hair root. The DIA3 isoenzymes were not detectable from hair samples of 1 week storage (Table 2).

This enzyme system appears to have a limited utility value in medicolegal practice, but may be a useful supplement for the grouping of hairs as long as they are fresh.

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