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Polymorphism of EsD by Isoelectric Focusing: Phenotyping in Human Tissues, Dental Pulps, Hair Roots, and Semen

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Summary. The polymorphism of EsD was investigated in tissues of various human organs, dental pulps, hair roots, and seminal stains by isoelectric focusing. The method yielded an excellent resolution of the isoenzyme components. The time limits of determination were: in organ tissues 3 weeks, in dental pulps 1 week, and in hair roots several days. The 7–1 type was less stable than the common types. Phenotyping was possible from fresh semen samples, but was unsuccessful from dried seminal stains after storage. The results show that the EsD typing by isoelectric focusing is of practical use for medicolegal individualization of organs, teeth, and hairs.

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

Zusammenfassung. Mittels Isoelektrofokussierung wurden die EsD-Typen aus verschiedenen menschlichen Organgeweben, Zahnpulpen, Haarwurzeln und Spermaspuren untersucht. Die Methode erbrachte eine ausgezeichnete Auftrennung der Isoenzymkomponenten. Die zeitlichen Nachweisgrenzen waren: an Organgeweben 3 Wochen, an Zahnpulpen 1 Woche und an Haarwurzeln mehrere Tage. Der Typ 7–1 war weniger stabil als die häufigen Typen. Die Typisierung gelang an frischen Spermaproben, aber an getrockneten Spermaspuren nach Lagerung nicht mehr. Die Ergebnisse zeigen, daß die EsD-Typisierung mittels Isoelektrofokussierung zur rechtsmedizinischen Individualisierung von Organen, Zähnen und Haaren von praktischem Nutzen ist.

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

Introduction

Since the discovery of EsD polymorphism by Hopkinson et al. (1973), the three common phenotypes (EsD 1, 2–1, and 2) controlled by two codominant alleles (EsD*1 and EsD*2) have been demonstrated not only in red cells, but also in bloodstains (Hayward and Bosworth 1975; Parkin and Adams 1975), various organ tissues (Hoste et al. 1977; Stöhlmacher and Haferland 1981; Kido et al. 1985), hair roots (Twibell and Whitehead 1978; Yoshida et al. 1979; Oepen et al. 1981; Lawton and Sutton 1982; Sutton and Bosley 1982; Gertler and Nagai 1983), and dental pulps (Henke et al. 1982) of human origin.

Recent isoelectric focusing studies have revealed the occurrence of EsD*5 allele in white people (Martin 1981) and of EsD*7 allele in Japanese (Nishigaki and Itoh 1984) with appreciable frequency, products both of which are difficult to identify by conventional starch gel electrophoresis. We have shown previously that the low voltage isoelectric focusing method developed by Yuasa et al. (1985a, 1985b) is simple, reliable, and suitable for phenotyping EsD in forensic stain analysis as well as in population studies (Komatsu 1985; Komatsu et al. 1985).

This paper describes further application of this isoelectric technique to the determination of EsD types from human tissues, dental pulps, hair roots, and seminal stains for medicolegal purpose.

Materials and Methods

Organ Tissues. The following tissues were obtained from 23 cadavers who were autopsied medicolegally within 48 h after death: spleen, pancreas, heart (cardiac muscle), liver, muscle (M. rectus abdominis), lung, skin (including adipose tissue), kidney, and brain. Heart blood was also obtained at autopsy from the same cadavers. Tissue sections (5 mm × 5 mm × 40 μm) were made using a cryostat (Tissue Tek II, Miles Laboratory, USA) at −20°C and attached on 5 × 5 mm filter paper (Toyoroshi No. 2, Tokyo, Japan). They were moistened with 10 μl 0.05 *M* dithiothreitol (DTT: Sigma Chemical Co., USA) for 30 min just before analysis.

Dental Pulps. Teeth were extracted from 35 patients who received treatment at the Dental Clinic of Yamanashi Medical University Hospital. Venous blood was also drawn from the same patients. 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 quantity (10–20 μl) of 0.05 *M* DTT.

Hair Roots. Human scalp hairs were plucked from 21 adult subjects of both sexes. Venous blood was also drawn from the same individuals. A single hair root bearing sheath cells was macerated on a hollowed glass plate in a minimum quantity (about 10 μl) of 0.05 *M* DTT and compressed with a glass rod to lyze the hair sheath cells.

Seminal Stains. Ejaculates were obtained by masturbation from 17 male volunteers. Venous blood was also drawn from the same men. The samples were pretreated with an equal volume of 0.05 *M* DTT for 15 min and subjected to electrophoresis. Seminal stains were made on filter paper (Toyoroshi No. 2), cut in 5 × 5 mm pieces and moistened with 10 μl 0.05 *M* DTT for 30 min just before analysis.

Ageing Studies. Samples of organ tissues, teeth, hair roots, and seminal stains were stored at room temperature and examined at different time intervals.

Isoelectric Focusing and Enzyme Staining. Isoelectric focusing was performed essentially by the method of Yuasa et al. (1985a) on polyacrylamide gel plates using Ampholine pH range 4.0–6.5 (LKB, Bromma, Sweden). Tissue sections and seminal stains were directly applied to the gel surface 2 cm from the cathode. Dental pulp lysates, hair root lysates, and semen samples were applied to the gel using 5 × 6 mm filter paper (Toyoroshi No. 2). After electrofocusing for 80 min at a constant voltage of 1,000 V, the gel was stained by the method of Hopkinson et al. (1973) using 4-methylumbelliferyl acetate (Koch-Light Laboratories Ltd., Berks, England) as substrate. The detailed procedure was described in our previous paper (Komatsu et al. 1985).

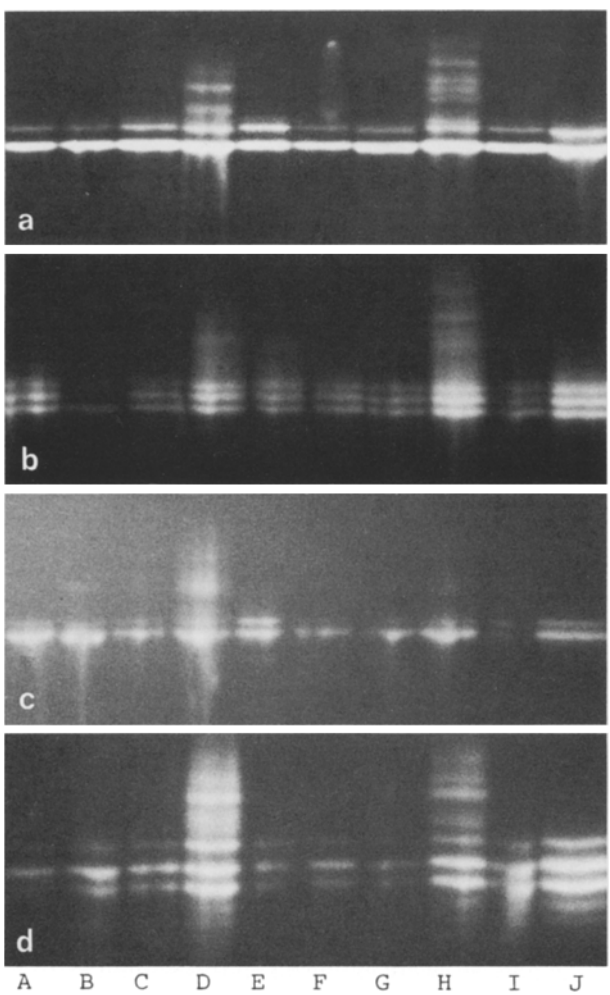

Fig. 1a–d. Isoelectric focusing patterns of EsD types in fresh organ tissues. **a** EsD 1; **b** EsD 2–1; **c** EsD 2; **d** EsD 7–1. *A* spleen; *B* pancreas; *C* heart; *D* liver; *E* muscle; *F* lung; *G* skin; *H* kidney; *I* brain; *J* red cell. The anode is at the *top*

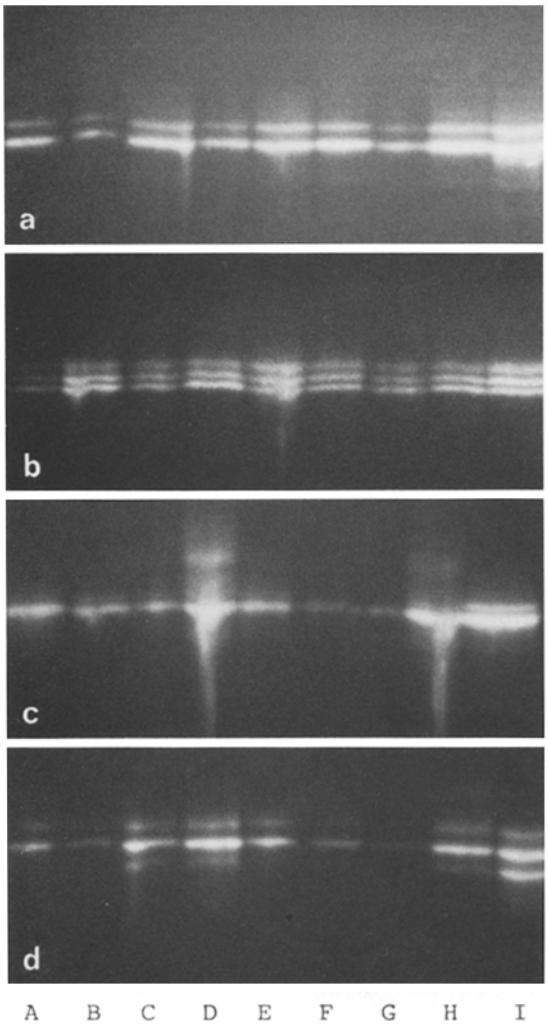

Fig. 2a–d. Isoelectric focusing patterns of EsD types in organ tissues stored for 2 weeks at room temperature. **a** EsD 1; **b** EsD 2–1; **c** EsD 2; **d** EsD 7–1. *A* spleen; *B* pancreas; *C* heart; *D* liver; *E* muscle; *F* lung; *G* skin; *H*, kidney; *I* red cell. The anode is at the *top*

Results and Discussion

Organ Tissues. As shown in Figs. 1 and 2, very clear EsD isoenzyme patterns were demonstrated from tissue sections of human spleen, pancreas, heart, liver, muscle, lung, skin, kidney, and brain by the present isoelectric focusing technique. The three common phenotypes were unmistakably determined from these tissues (except brain) stored for up to 3 weeks, while the EsD 7–1 type for up to 1 week. The results are summarized in Table 1, which indicates that the method is quite suitable for grouping organ tissues even if they are decomposed.

Table 1. Positive results for the determination of EsD types from tissues of various human organs

Phenotype	No. tested	Period of storage	Tissue								
			Spleen	Pancreas	Heart	Liver	Muscle	Lung	Skin	Kidney	Brain
1	7	48 h	7	7	7	7	7	7	7	7	7
		1 week	7	7	7	7	7	7	7	7	7
		2 weeks	7	7	7	7	7	7	7	7	
		3 weeks	7	7	7	7	7	7	7	7	
2–1	12	48 h	12	12	12	12	12	12	12	12	12
		1 week	12	12	12	12	12	12	12	12	12
		2 weeks	12	12	12	12	12	12	12	12	
		3 weeks	12	12	12	12	12	12	12	12	
2	2	48 h	2	2	2	2	2	2	2	2	2
		1 week	2	2	2	2	2	2	2	2	2
		2 weeks	2	2	2	2	2	2	2	2	
		3 weeks	2	2	2	2	2	2	2	2	
7–1	2	48 h	2	2	2	2	2	2	2	2	2
		1 week	2	2	2	2	2	2	2	2	2
		2 weeks	1	0	1	1	1	1	1	2	
		3 weeks	0	0	1	1	1	1	0	2	

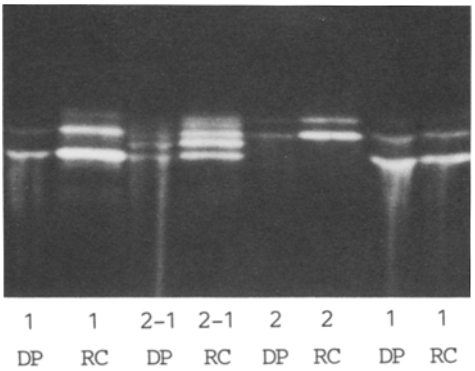

Fig. 3. Isoelectric focusing patterns of EsD types in dental pulps. *DP* dental pulp; *RC* red cell. The anode is at the *top*

Table 2. Positive results for the determination of EsD types from dental pulps

Phenotype		Fresh	1-week-old
	No. tested	28	7
1		14	2
2–1		12	4
2		2	1

The enzyme typing is particularly useful in mass disasters, such as airplane crashes and explosions when piecing together dismembered corpses.

The present results for the determination limits are almost similar to those obtained by our previous starch gel electrophoretic experiments (Kido et al. 1985) comparable to this work. However, isoelectric focusing provides an excellent resolution of EsD isoenzymes and furthermore permits detection of the product of EsD*7 allele. This product seems less stable than the product of common EsD*1 and EsD*2 allele, as recognized earlier also in the examination of bloodstains (Komatsu et al. 1985). Nevertheless, the EsD*7 allele is of great significance because it occurs with appreciable frequency in Japanese (our data: 0.0103, Komatsu et al. 1985). We have recently experienced a fatal case of traffic accident where the enzyme typing by isoelectric focusing has been successfully applied to the assignment of a piece of muscle tissue found at the bottom of a suspected car to the victim, both being typed as EsD 7–1 (Oya et al. 1986).

Dental Pulps. EsD isoenzymes in dental pulp tissues stained as intense as those in the corresponding hemolysates (Fig. 3), and reliable phenotyping was possible not only from fresh samples but also from 1-week-old samples (Table 2). The banding patterns are apparently much clearer than those initially demonstrated by Henke et al. (1982) using starch gel electrophoresis. The EsD typing

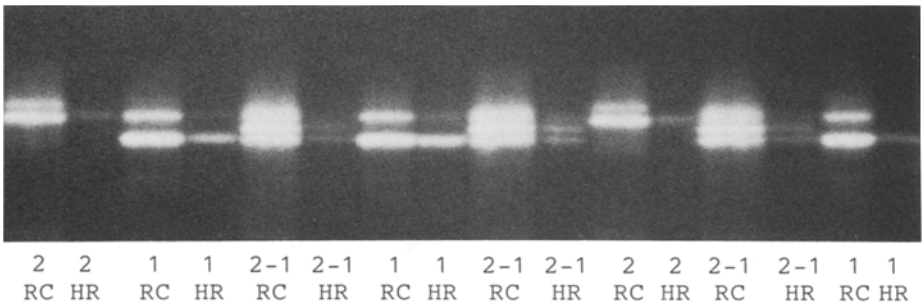

Fig. 4. Isoelectric focusing patterns of EsD types in hair roots. *HR* hair root; *RC* red cell. The anode is at the *top*

Table 3. Positive results for the determination of EsD types from a single hair root

Phenotype	No. tested	Period of storage (days)							
		Fresh	1	2	3	4	5	6	7
1	7	7	7	7	7	6	3	2	2
2–1	8	8	8	7	7	4	2	0	
2	4	4	4	3	1	1	0		
7–1	2	2	2	1	0				

Table 4. Positive results for the determination of EsD types from semen and seminal stains

Phenotype	No. tested	Semen	Seminal stain	
			1-day-old	2-day-old
1	8	8	3	2
2–1	9	9	0	
2	0			

by isoelectric focusing can therefore be a powerful means for personal identification of the teeth.

Hair Roots. The EsD patterns in hair roots were distinct, but faint as compared with those in the corresponding hemolysates (Fig. 4). The intensity of the isoenzyme bands depended on the amount of sheath cells present in each hair root. Phenotyping was possible within a few days after extraction of the hair (Table 3). Thus the isoelectric focusing method proposed by Yuasa et al. (1985a) has been confirmed to be feasible and reproducible for EsD typing from a single hair root. It may be advisable that the samples be examined as soon as they are collected in forensic casework.

Seminal Stains. The present isoelectric focusing technique allowed EsD typing from fresh semen samples, which had not succeded by conventional electrophoresis (Oepen et al. 1980). The types in semen agreed well with those in the corresponding red cells. However, activity was so weak that phenotyping was not reliable without control samples. The results are shown in Table 4. This polymorphism seems of limited value for medicolegal use since typing was unsuccessful from dried seminal stains after storage.

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