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THE ESTERASE D POLYMORPHISM: ANALYSIS OF ESTERASE D 7 BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

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Summary Qualitative and quantitative differences between the EsD 7 and EsD 1 subunits are analysed by two-dimensional gel electrophoresis. The molecular weight of the EsD 7 subunit is slightly smaller than that of the EsD 1 subunit. However, the pI of the EsD 7 subunit is indistinguishable from that of EsD 1 subunit. In the heterozygote with EsD 7-1, the amount of the EsD 7 subunit is very similar to that of the EsD 1 subunit. The results indicate that EsD 7 is a molecular weight variant and coded for by a new allele, EsD^7, distinct from EsD^1 at EsD locus. The data also suggest that the amount of the EsD^7 product is similar to that of the EsD^1 product but that the specific activity of EsD 7 is very low compared with that of EsD 1.

INTRODUCTION

Esterase D (EsD) is one of the abundant enzymes in a variety of tissues, and shows a genetic polymorphism in all the population examined. In Japanese, the heterozygosity of the EsD locus is high (Ishimoto *et al.*, 1974; Nishigaki *et al.*, 1983). Furthermore, it has been shown that the locus for EsD is closely linked to the retinoblastoma locus 1(*Rb-1*) (Sparkes *et al.*, 1980; 1983). Therefore, the *EsD* locus is a useful genetic marker.

Since two common alleles, EsD^1 and EsD^2, were discovered by Hopkinson *et al.* (1973), rare alleles, EsD^3, EsD^4, EsD^5 and EsD^6, and silent alleles (Marks *et al.*, 1977; Sparkes *et al.*, 1979; Koziol and Stepien, 1980), have also been reported by the use of starch-gel electrophoresis (Bender and Frank, 1974; Berg *et al.*, 1976; Radam *et al.*, 1980) or agarose-gel electrophoresis (Martin, 1979; Olaisen *et al.*, 1981). In addition, Nishigaki and Itoh (1984) have shown the existence of two EsD^1 products, designated EsD 1 and EsD 7, using isoelectric focusing (IEF). The gene frequencies of

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EsD^1 and *EsD*^{7} are 0.630 and 0.009, respectively, in a Japanese population and the enzyme activity of EsD 7 is one fourth the EsD 1 activity (Nishigaki and Itoh, 1984).

Recently, we have described that the polymorphism of EsD can be easily analysed by two-dimensional gel electrophoresis (two-D gel electrophoresis) and that the subunits of EsD 1 and EsD 2 have very similar molecular weight (mol. wt.) but different pI values. In this study, to examine qualitative and quantitative differences between the EsD 7 and EsD 1 subunits, the EsD 7 subunit is identified and compared with the EsD subunit by two-D gel electrophoresis.

MATERIALS AND METHODS

Blood samples were washed three times with phosphate buffered saline and packed erythrocytes were lysed by freezing and thawing three times. Two-D gel electrophoresis of the erythrocyte lysate was carried out by the method developed by O'Farrell (1975) with some modifications (Kondo *et al.*, 1984). The proteins were visualized by the silver staining method described by Merril *et al.* (1981). The EsD 7 phenotype was determined by IEF (Nishigaki and Itoh, 1984).

RESULTS AND DISCUSSION

Figure 1 shows the phenotypes of EsD 2-1, EsD 7-1 and EsD 7-2 in the two-D gel electrophoresis pattern. The EsD subunits (polypeptides) are indicated by bold arrows. Actin, carbonic anhydrase (CA), catalase (CAT), and 35k and r34k polypeptides which are shown by thin arrows are useful reference markers for the identification of the EsD subunits and the determination of the phenotypes of EsD in the two-D gel electrophoresis pattern (Kondo *et al.*, 1984). The subunits of EsD 1 and EsD 2 differ in pI from each other but very similar in mol. wt., as previously reported (Kondo *et al.*, 1984) (Fig. 1A). The EsD phenotypes have been examined in more than 150 unrelated individuals by two-D gel electrophoresis and the polypeptide marked EsD 7 in Fig. 1B and 1C has been observed only in the individuals who are determined to have EsD 7 by IEF. Therefore, the polypeptide marked EsD 7 is considered to be the EsD 7 subunit. Thus, the EsD 7 subunit was identified on the two-D gel.

The mol. wt. of the EsD 7 subunit is slightly smaller than that of the EsD 1 subunit. However, the pI of the EsD 7 subunit is almost identical with that of the EsD 1 subunit under the conditions of the two-D gel electrophoresis system, in spite of the fact that EsD 7 and EsD 1 are separated from each other by IEF (Nishigaki and Itoh, 1984). The result suggest that EsD 7 is a molecular weight variant.

The amount of the EsD 7 subunit is very similar to that of the EsD 1 subunit in the heterozygote with EsD 7-1. Since the enzyme activity of EsD 7 is one fourth the EsD 1 activity (Nishigaki and Itoh, 1984), the data suggest that the amount of the *EsD*^{7} product is similar to that of the *EsD*^{1} product but that the specific activity of EsD 7 is very low compared with that of EsD 1.

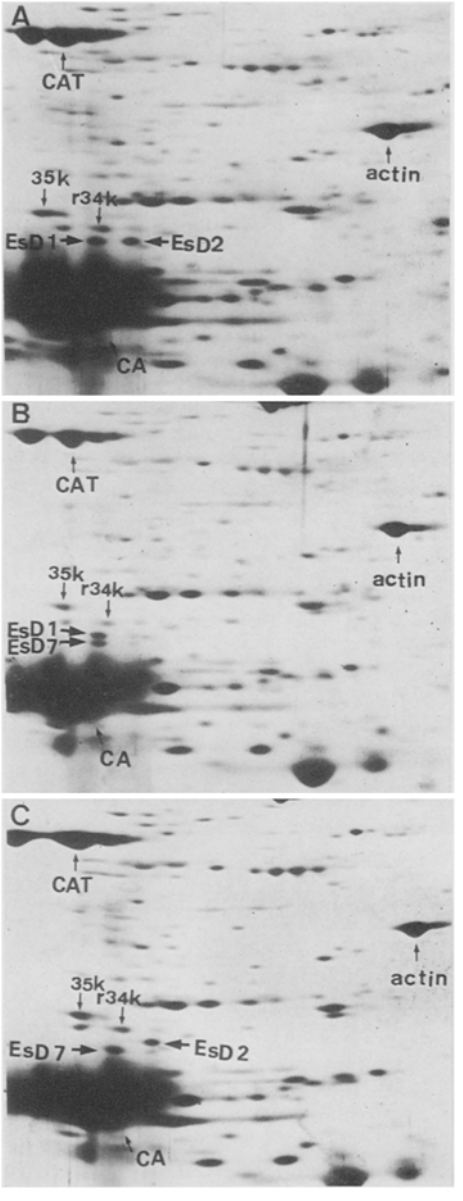

Fig. 1. Three phenotypes of EsD in the two-D gel electrophoresis pattern of erythrocyte lysates. A: EsD 2-1, B: EsD 7-1, C: EsD 7-2. Isoelectric focusing was from left to right and mol. wt. separation from top to bottom. The EsD 1, EsD 2 and EsD 7 subunits are shown by bold arrows. Other polypeptides indicated by thin arrows are the reference markers for the identification of EsD subunit and the determination of the EsD phenotypes.

The results presented in this paper indicate that EsD 7 is coded for by a new allele, EsD^7 distinct from EsD^1. For the EsD polymorphism, atypical segregation and the presence of silent alleles have been reported (Marks *et al.*, 1977; Sparkes *et al.*, 1979; Koziol and Stepien, 1980). EsD 7 may be related with some of these observations, because of it's mobility similar to that of EsD 1 in the convensional electrophoresis system and it's low specific activity.

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